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A SARCOMA IN A GUINEA PIG

F. C. WOOD, M.D.

(From the George Crocker Special Research Fund, Columbia University, New York.)

On May 19, 1915, the George Crocker Special Research Fund received, through the kindness of the staff of the Research Laboratories of the Department of Health of the City of New York, an adult female guinea pig, weighing 710 gm. and bearing a tumor on the right foreleg. The growth, which was pear-shaped, was

6 cm. long, 5 cm. broad at the widest portion, and 2 cm. at the narrowest; it was hard and not attached to the skin. At operation, under ether anesthesia, the tumor was found to be so intimately connected with the bone that amputation of the leg was required for its extirpation. The neoplasm weighed 60 gm. and was firm in consistency and whitish in color; it appeared to spring from the ulna.

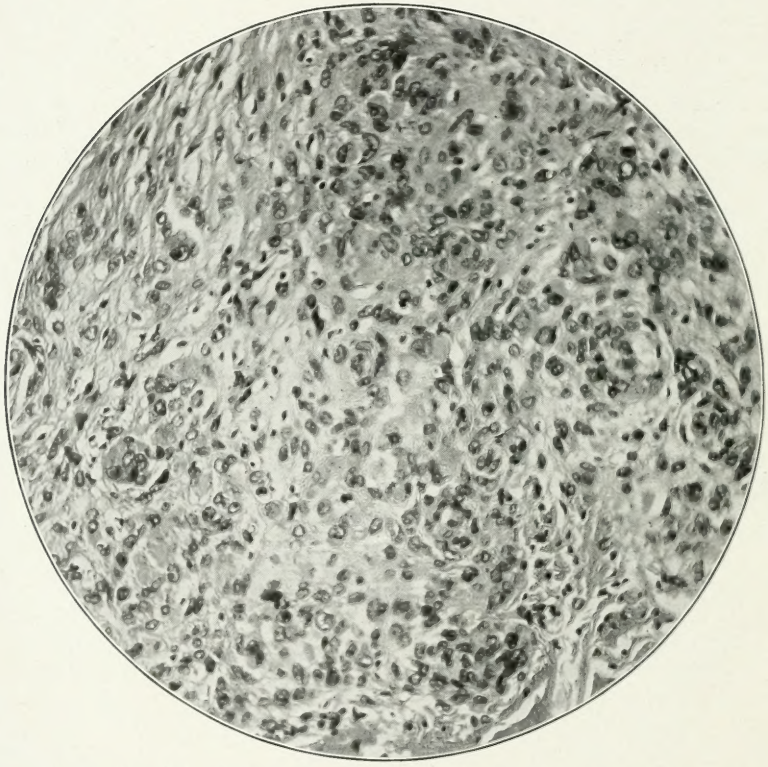

FIG. 1. Section of primary tumor showing richly cellular area. × 200.

Six grafts were inoculated into the pig herself, and the remainder of the mass was preserved for transplantation and for histological study. Unfortunately, the pig died during the following night; an autopsy showed nothing except an ovarian cyst and pregnancy. No gross metastases were discovered after a most careful search.

The tumor was transplanted into 569 young guinea pigs, weighing from 180 to 200 gm., 0.01 gm. in the right and 0.02 gm. in the left groin. Tumors began to develop in these pigs on July 7, 1915, forty-nine days after inoculation, and continued

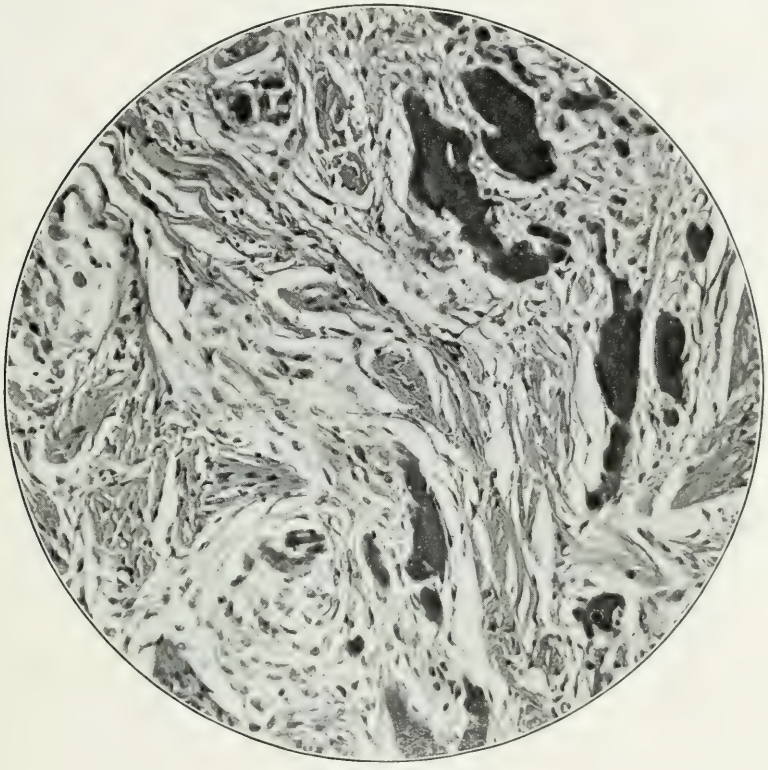

FIG. 2. Section of primary tumor showing areas of calcification. × 200.

to appear up to December 15, or 210 days after inoculation. More tumors were obtained from the 0.02 gm. dose than from the smaller, though not a few developed from the 0.01 gm. grafts; in several pigs both grafts gave rise to tumors.

Of the 569 pigs, 34 have developed tumors varying from the size of a small green pea to that of a hen's egg. A few of the smaller nodules have disappeared, but the great majority are still growing progressively. The percentage of "takes" might have

been larger had not 374 of the pigs been lost in an epidemic. The basis for calculation is, therefore, 34 positives in a total of 195 pigs, or 17 per cent. All the growths so far examined have been replicas of the spontaneous neoplasm.

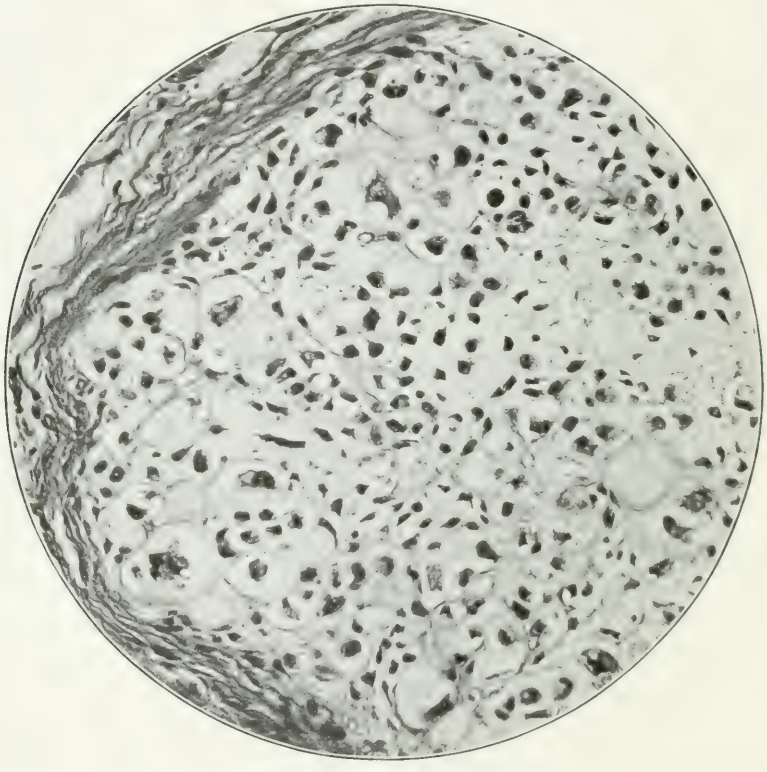

FIG. 3. Section of primary tumor showing cartilaginous area. × 200.

The second generation has not proved so adaptable. By the first transplantation, September 30, 1915, one receding tumor was obtained in 109 pigs, 58 of which have survived until now. The second, November 26, 1915, gave one progressively growing neoplasm in 138 pigs, 125 of which are still living. A third series, transplanted December 1, 1915, has so far produced no tumors in 250 pigs, 210 of which are still living. Other series, comprising several hundred animals, have been laid down too recently for tumors to appear.

On microscopical examination, the tumor was found to be a fibrosarcoma. The proportion of fibrous tissue varied in different parts of the growth (Fig. 1), the more vascular areas being more cellular. Some portions of the tumor were somewhat edematous

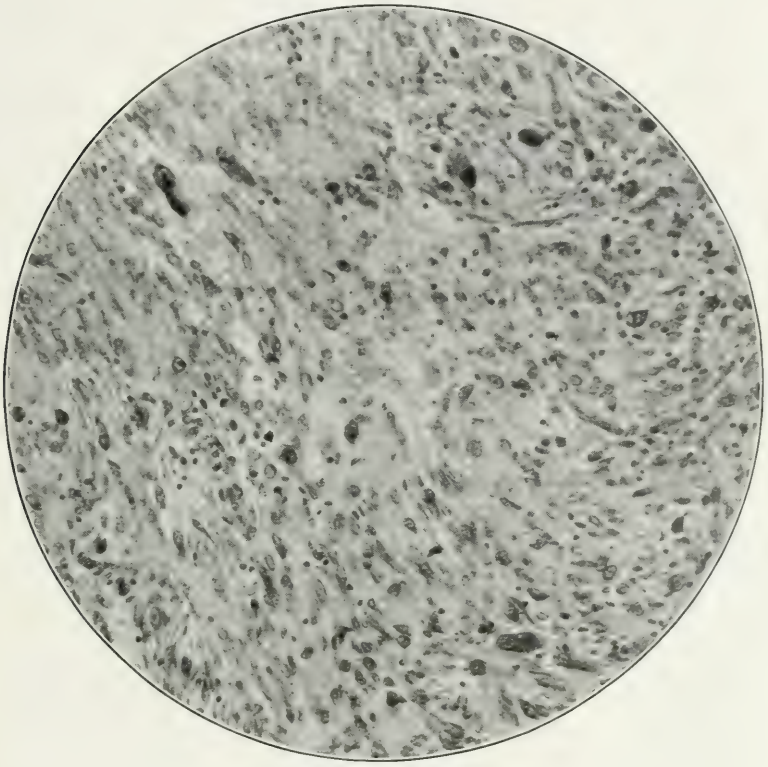

FIG. 4. Section of tumor in first generation. × 200.

and others were very firm. The tumor involved the periosteum and had invaded the medullary cavity of the bone. Scattered throughout the connective tissue near the bone were a number of calcified areas (Fig. 2), and there was one spot which suggested cartilage (Fig. 3), but might be entirely due to trauma. No cartilage was found in the first generation of transplanted tumors; the type of growth remained the same except that it was perhaps a little more cellular (Fig. 4) and contained more cells with ir-

regular nuclei. Mitotic figures were not infrequent both in the spontaneous growth and in the first generation of transplants.

No metastases were discovered in sections from the heart, lungs, liver, spleen, pancreas, kidneys, adrenals, and ovaries.

Only two other tumors of the guinea pig have, so far as is known, been reported; one by Murray[1] and the other by Dickson.[2] The latter could not be transplanted. Murray's tumor was a very slow growing fibrosarcoma, closely resembling the one herein described.

Discussion:

Dr. Levin: Is it possible that there is some relationship between the fact that guinea pigs are especially sensitive to the introduction of foreign proteids and the fact that the occurrence of malignant tumors in these animals is rare?

Dr. Zinsser: As a matter of fact it is difficult to produce anaphylaxis in mice, and Ritz and Sachs believe that the complement of mice contains only the mid-piece. However, it would seem to me that this could hardly be taken as support of any such bold theory applied to cancer. In fact, we know very little about anaphylaxis in mice.

Dr. Wood: Dr. Levin's anaphylactic theory does not agree with the fact that rabbits do not often have tumors, and at the same time are not very sensitive to anaphylaxis.

THE HISTOPATHOLOGY OF DISEASES OF THE APPENDIX

ELI MOSCHCOWITZ, M.D.

The purposes of this report are two: first, to attempt to clarify some current ideas concerning the pathology of appendicitis; and second, to demonstrate and establish definite criteria by which disease of this organ may be recognized. Pioneer work in this field was performed by Aschoff and since the publication of his work but little has been added to the subject. The present report is the result of the study of a large series of appendices, about 1,500 in all. The various diseases will be discussed in order, beginning with acute inflammations.

The earliest lesion in acute appendicitis is quite characteristic.

[1] Murray, J. A., Jour. Path. and Bacteriol., 1916, xx, 260.

[2] Dickson, E. C., *Proc. Soc. Exper. Biol. and Med.*, 1915, xiii, 26.

It begins with a loss of epithelium and the formation of an exudate which appears first at the base of each crypt of the appendix and extends from there into the lumen. At the same time, there is an infiltration of polymorphonuclear leucocytes throughout all the coats, and a localized peritonitis. Every case in this series shows a localized peritonitis, but it must be admitted that in no case was the appendix removed less than 11 hours after the onset of symptoms. As the acute process advances, there is greater and greater destruction of mucosa, and in the more advanced stages the lumen is completely filled with exudate. The infiltration of the muscular coats also becomes more intense until it is finally impossible to recognize any of the histological components of the appendix. Ultimately, necrosis supervenes, resulting in so-called gangrene. The lymphatic apparatus of the appendix has a peculiar immunity from the destructive process. The extent of the acute lesion corresponds in general to the duration of the illness.

The following conclusions may be drawn from the study of the pathological anatomy of acute appendicitis: *first*, there is every ground for presuming that the infection is enterogenous rather than hematogenous in origin; *second*, the lesion is not a catarrhal inflammation in the usual meaning of the term; *third*, the lesion is the result of bacterial infection and begins in the crypts because stagnation is more apt to occur here; *fourth*, the pathological lesion corresponds fairly accurately to the duration of the illness; *fifth*, the lesion is a membranous and suppurative inflammation; a membranous inflammation is in fact the usual type in all acute infections of the mucous membranes; *sixth*, the best term by which to designate the lesion is "acute appendicitis." All other terms, such as gangrenous, ulcerative, catarrhal, etc., should be discarded.

In studying the pathological anatomy of acute perforation of the appendix it is found that perforation usually occurs only in the later stages of the malady. Rupture is apparently due to necrosis with increased tension of the intra-appendicular contents. It is unlikely that thrombosis of the mesentery is an important factor in causing perforation.

The majority of empyemata of the appendix seem to be due rather to the accumulation of purulent material in a previously dilated portion of the appendix, extending from an adjacent acute lesion of the organ, rather than to a primary acute inflammation at the site of the empyema with secondary dilatation. The second chain of events may occur but it is exceptional.

In healing appendices one finds in some specimens an ulcer which shows beginning ingrowth of epithelium from the adjacent normal mucosa. In other specimens there is complete destruction of the mucosa with replacement by granulation tissue. There are concomitant healing processes in the other coats of the appendix. The course of events is the following: the exudate or membrane breaks down, becomes necrotic and leaves an ulcer covered with granulation tissue. If the mucosa has been completely destroyed there is complete obliteration of the lumen. If the mucosa has been only partially destroyed a stricture is formed. At the same time, the formation of new connective tissue in the muscular coats leads to two changes—deformity and separation of the muscular fibers. The peritoneal coat may undergo thickening and adhesions may be formed.

The healed or chronically diseased appendices studied were of two types, those showing stricture and those showing obliteration. In addition these appendices showed the following lesions which are characteristic of chronic appendicitis: *first,* absence of mucosal crypts; *second,* widening of the submucous connective tissue; *third,* diminution, or even complete absence of the lymphoid tissue; *fourth,* infiltration and deformity of the muscular coats; and *fifth,* thickening, increased density and deformity of the peritoneal coat. All of these conditions result from primary acute lesions. It is conceivable that if the acute process were arrested early there might be a complete restitution to the normal. Such an occurrence did not take place, however, in any of the cases studied, and the conclusion seems warranted that an appendix which is anatomically normal has never been the seat of an acute attack.

The lesion of chronic appendicitis is in every sense an end

product and not a progressive and continuous inflammatory process. This study has shown no lesion which could be classed as a "chronic catarrhal appendicitis." The chronic condition is always the sequel of an acute attack, even though clinically there be no history of such an attack.

A number of lesions were observed which simulate appendicitis:

(*a*) Submucous hæmorrhages in the appendix. These are due to trauma at operation.

(*b*) Coproliths. These are probably not a direct cause of an acute attack. They only influence the process by interfering with the drainage of the organ.

(*c*) Lymphoid hyperplasia of the appendix. This lesion is almost always associated with a status lymphaticus.

(*d*) Oxyuris in the appendix. This is an exceedingly common finding. In no case could an invasion of the coats of the appendix by the worm be observed. As a rule the presence of oxyuris gave no symptoms.

(*e*) Trychocephalus dispar.

(*f*) Decidua of the appendix. The decidual cells are found in the subperitoneal connective tissue. At least two dozen appendices, removed in the course of operations for ectopic pregnancy, were examined, but this lesion was observed only once. It should be regarded as merely another evidence of the widespread decidual reaction in the pelvic connective tissue that occurs normally in pregnancy.

(*g*) Intra-appendicular mucocele. The appendix shows evidence of a chronic inflammation. The lesion is characterized by a complete conversion of the appendicular epithelium into the mucous or goblet type. These appendices sometimes rupture, leading to the strange phenomenon known as muco-peritoneum.

(*h*) Extra-appendicular mucocele. This is a small connective tissue vesicle on the peritoneal surface filled with mucus. The genesis of mucoceles is not clear but it may be related to a previous perforation of the appendix.

(*i*) Diverticulum of the appendix. Two cases were observed.

(*j*) Carcinoma of the appendix. Three types are found, scirrhus, medullary and adeno-carcinoma. The lesions may be found in association with acute appendicitis. Carcinoma of the appendix is comparatively non-malignant both from the clinical and the pathological point of view.

CONCLUSIONS

I. The pathological lesion of acute appendicitis represents a suppurative process from the very beginning. The earliest lesion is as characteristic as the primary lesion of syphilis, and all the subsequent stages of the disease within the organ are directly traceable to the spread and development of this lesion. There is no pathological evidence whatever that an acute catarrhal inflammation of the appendix occurs.

II. The changes grouped under the name chronic appendicitis —stricture, obliteration, etc.—are the result of the healing of the acute lesion. According to this interpretation, chronic appendicitis is not a continuous and progressive inflammation, but an end product. There is no pathological evidence that involution of the appendix or chronic catarrhal inflammation of the appendix occur.

III. The only justifiable classification of inflammations of the appendix, therefore, is the following:

(*a*) Acute appendicitis.
(*b*) Healing or subacute appendicitis.
(*c*) Healed or chronic appendicitis.

IV. An acute localized peritonitis with the formation of fibrin and limited to the site of the lesion is always present in acute appendicitis as early as 12 hours after the onset, and perhaps earlier, consequently the absence of a localized peritonitis on gross inspection of suspected cases is in itself indicative of absence of acute appendicitis.

V. In addition to obliteration and stricture, attention is called to two new pathognomonic signs of chronic appendicitis, which are constant and easily recognized, namely, absence of mucosal crypts and marked widening of the submucous connective tissue zone

The latter change is especially easy to detect upon cross section of the organ, and is valuable as the simplest indication of the presence or absence of a chronic appendicitis.

VI. Cross-sections of the appendix at various levels are far preferable to longitudinal sections for the study of pathological changes.

Discussion:

Dr. Larkin: It seems to me that Dr. Moschcowitz has given us a great deal of cause for reflection in presenting Aschoff's idea of appendicitis. This, to my mind, is not in accord with many surgeons' ideas as to what appendicitis is or is not. To those of us who have been examining appendices for years the pictures presented are familiar. Dr. Moschcowitz has put a great deal of study into this paper, particularly into the pathological anatomy, and I think he is to be congratulated. There is one picture which he has presented this evening which has an important medico-legal bearing. I refer to the section of an appendix in which decidual cells are seen beneath the peritoneum. This observation was first made by Pick, who discovered beneath the peritoneum of the uterus in Douglas' cul-de-sac several small nodules, which, upon microscopic examination, proved to be decidual cells. This was a novel find at the time, but it is now being gradually recognized as of great importance in its connection with pregnancy. It may be of great medico-legal interest, especially where the uteri are cleaned out so completely by an abortionist as to leave no vestige of placental or decidual remains. In examining a large number of uteri after abortion and pregnancy, I have been surprised at the frequency of decidual remains after careful microscopic examination, which were identical with the picture Dr. Moschcowitz presented this evening. Perhaps all of us cannot agree with his classification, several points of which he mentions as having been accepted by most pathologists. His classification, in my opinion, seems rather radical.

Dr. Pappenheimer: Because after twelve hours lesions are found in the mucosa, it does not, I believe, follow that the infection was originally enterogenic rather than through the blood stream. Dr. Moschcowitz has pointed out the coexistence of a local peritonitis at this time, indicating that the distribution of the organisms through the wall of the appendix is quite widespread. In the case of early tuberculosis of the Fallopian tubes, in which the infection is unquestionably hematogenous, the lesions are first seen in the mucosa and rapidly lead to a destruction of the overlying epithelium. It would seem to me, therefore, that Aschoff's views, which Dr. Moschcowitz seems to have adopted, while probably correct for most cases, can hardly be considered as absolutely substantiated. I should like to ask also whether Dr. Moschcowitz believes that senile involutionary changes in themselves may lead to obliteration of the appendix?

Dr. Wood: I recently examined an appendix which had been removed from a patient suffering from an acute attack of appendicitis with fever and a leucocyte count of 26,000. The whole lumen of the organ was changed into

fibrous tissue and yet there was an acute suppurative process in the subserosa. The patient denied any previous attack. It is rather difficult to put such appendices into an ordinary classification.

Dr. Lambert: I was rather disappointed to hear Dr. Moschcowitz say that he regarded the so-called carcinomata of the appendix as true cancers. It seems to me that the term carcinoma should not be applied to any but malignant epitheliomatous growths and that the only safe criterion of malignancy is the behavior of the cells which compose the tumor. The mere fact that cells are arranged in solid nests does not afford a sufficient basis for calling them carcinoma cells. That the majority of these tumors of which Dr. Moschcowitz has reported several typical examples show little tendency to invasive growth and do not metastasize is well known. The cells, furthermore, as he has pointed out, do not resemble those seen in most malignant tumors. They are small and usually show deeply staining nuclei. One rarely finds mitotic figures among them. It appears to me, therefore, that we are not warranted in applying the term carcinoma to such growths.

Dr. Ewing: I agree with Dr. Larkin that the reader of the paper deserves much credit for bringing out so much of interest in this important subject. I would like to ask if the designation of chronic catarrhal appendicitis does not apply to mucocele. That seems to me legitimate. I was not aware that they filled the abdominal cavity with mucus. I thought that was from a tumor of the peritoneum. Most of us would agree with the general statements made, but perhaps there is too great finality about some of the conclusions. I do not think you can always say of a lesion that it does this or that invariably. That is a hazardous view to take. Dr. Delafield recognized several types of appendicitis, the catarrhal, purulent, exudative, necrotic and gangrenous. I confess I have been teaching that classification several years to students. I have seen sections which reflect these types. I don't always find peritonitis on the surface. In very acute stages peritonitis occurs. I have seen what I would regard as early gangrenous appendicitis. This, I think, is determined by extensive thrombosis of the vessels. Generally speaking, Dr. Moschcowitz is correct as regards the great majority of surgical specimens. One finds catarrhal enteritis and catarrhal colitis, so also appendicitis. Surgeons don't see these but I think they occur.

I cannot refrain from discussing Dr. Lambert's remarks on the cancer question. It is known that these tumors differ from all other forms of carcinomata. In different organs cancers do not act alike. There are some appendix carcinomata which do metastasize and do infiltrate, but not excessively. They lack the complete clinical picture of cancer. So do rodent ulcers. If the appendix types had ten or fifteen years to develop they might express their capacity for malignancy.

Dr. Levin: I want to express my appreciation of the wonderful erudition of Dr. Moschcowitz's paper. It is unusual in these days to find a man willing to study a subject for six years until he gets ready to say something. One word in regard to cancer of the appendix—during my practice I can remember two clinical cases where the condition began as an acute appendicitis with all the clinical symptoms. The patients refused operation. Ulti-

mately operation showed a carcinoma of the cæcum. I believe that carcinoma of the appendix is so frequently found to be circumscribed because the clinical symptoms appear sooner than in almost any other malignant tumor.

Dr. Moschcowitz: In answer to Dr. Pappenheimer's remarks, the reason I believe appendicitis is always enterogenic in origin is because the lesion always begins in the mucosa and is associated with a distinct loss of epithelium. Moreover, I have never seen a focal infection in the walls of the appendix unassociated with a mucosal lesion.

In regard to Dr. Wood's case the question of the genesis of certain obliterated appendices has puzzled me greatly. In the vast majority of instances a previous history of one or more attacks of appendicitis can be elicited, especially after a careful cross-examination. In a few no history of anything resembling a previous attack is obtainable. However, from a pathological point of view, I can not conceive that extensive obliteration of the appendix could occur without previous destruction of the mucosa.

In answer to Dr. Ewing I agree and have so stated in this paper, that mucoceles are consequent to chronic inflammations of the appendix. I would assure Dr. Ewing that extensive collections of mucus can collect in the peritoneal cavity from simple rupture of mucoceles of the appendix or old pus tubes. I have myself seen such an instance. It seems impossible to obtain a precise difinition of the term "catarrhal inflammation." As I understand it—and the majority of pathologists whom I have questioned agree—a "catarrhal inflammation" of a mucous membrane is characterized by congestion and exudation of mucus.

Dr. Ewing: A catarrhal inflammation is marked by an exudate of serum, pus, or mucus, thrown off a surface by desquamation of the lining cells.

Dr. Moschcowitz: Most pathologists do not agree with the view of Dr. Ewing that a catarrhal inflammation is associated with pus. They class such inflammations as suppurative.

Dr. Ewing: Volkmann accepts that definition.

Dr. Moschcowitz: Others do not, and especially Aschoff. Dr. Ewing criticised some of the statements in the paper as perhaps too categorical. I have only made such statements categorical, as I believe my observations demanded. In regard, for instance, to the question of localized peritonitis in acute appendicitis, my observations upon many hundreds of appendices removed during an acute attack entitle me to say definitely that a localized peritonitis is always present in every case of acute appendicitis. I have never seen an exception to this rule.

In regard to thrombosis of the mesentery, I am unwilling to state how often it occurs, because I have been unable to determine whether the thrombosis was ante- or post-operative.

STUDIES ON TREPONEMA PALLIDUM AND SYPHILIS

HANS ZINSSER, M.D., J. G. HOPKINS, M.D. AND MALCOLM McBURNEY, M.D.

(*From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University, New York City.*)

(*a*) FLUCTUATIONS IN VIRULENCE OF INDIVIDUAL STRAINS AND COMPARATIVE VIRULENCE OF DIFFERENT STRAINS OF TREPONEMA PALLIDUM IN RABBITS

Incident to other laboratory experiments on treponemata several strains have been kept going in rabbits and the fluctuations in virulence, incubation time, character of lesions, etc., observed.

Five strains have been kept alive through from ten to more than twenty generations in rabbits. Four of these were obtained from chancres and mucous patches; the fifth is a strain with which Dr. Nichols worked and which he very kindly sent to this laboratory. It has appeared that fluctuations in virulence, differences in incubation time and differences in the character of the lesions obtained have depended, not on the special virulence of any particular strain, nor upon the number of passages through rabbits, but upon various other factors:

1. The character of the rabbit lesion from which the inoculation was made; young, fresh, diffuse lesions giving a higher degree of virulence and a shorter incubation time than older, more indurated lesions.

2. The size of the testicles of the rabbits which were inoculated; the higher percentage of takes with a shorter incubation period being obtained in rabbits with well-developed testicles.

3. The technique of the inoculation, a thorough maceration of the tissue to be inoculated and a thorough injection of the material all through the testicle tending to decrease the incubation time and increase the virulence.

4. Absence of delay between removal of lesion and inoculation into fresh rabbits.

5. The character of the lesion has seemed to depend considerably on whether it developed in the testicle or in the firmer tissues of the sac.

Upon these various factors have seemed to depend all changes in virulence, incubation time, or character of the lesion. All kinds of lesions and variations in incubation time have occurred in each one of the five strains and frequently in the same generation, while no increase in virulence has been observed after repeated passage through rabbits. Noguchi has described three types of treponemata which differ in thickness and in their virulence for rabbits. We have been unable to identify these in any particular kind of lesion or any particular strain. In fact, we have seen what appear to be thick and thin types in the same preparation.

In regard to the generalization of the disease in rabbits, we have noticed three cases of keratitis, each one occurring in a different strain. Aside from these and a few minor lesions, such as involvement of the prepuce and in one case demonstration of the treponemata in the peritoneal cavity, we have noticed no tendency to generalization in any of the strains.

(*b*) GROUP AGGLUTINATIONS OF TREPONEMATA IN PALLIDUM ANTISERUM

A year ago we reported the observation of agglutinative substances for *Treponema pallidum* from cultures, in the serum of rabbits immunized to these cultures. Following this observation we wished to determine the specificity of the reaction, whether group reactions might be expected in the blood of individuals infected with species of treponemata other than pallida, and also whether the reaction might be used for the identification of cultures. In carrying out these tests we have used the serum of immunized rabbits and of an immunized sheep. The material for injection has been obtained by taking cultures in sheep serum broth to which kidney tissue had been added, at their height of growth (from 3 to 6 weeks) centrifuging the treponemata from the growth at high speed, resuspending the sediment in salt solu-

tion and killing the organisms by heating at 56° for half an hour. In case precipitate was present in the suspensions, this was removed by short centrifugation at low speed. The sheep at the time these experiments were made had received 22 injections over a period of eight months of from 10 to 25 c.c. each of concentrated *pallidum* suspension. One of the rabbits (No. 624) received 30 injections over a period of 12 months of about 1 c.c. each. Another rabbit which had received only 9 injections over a period of two months showed nearly as powerful agglutination.

In carrying out the tests, suspensions of treponemata were used prepared like those used in immunization, except that the cultures were grown in ascitic broth instead of in sheep serum broth to avoid confusion from protein anti-protein reactions. The suspensions were freed as nearly as possible from precipitate and were used unheated. In our first experiments the macroscopic reaction was so striking that we depended entirely on reading with the naked eye. We soon found, however, that occasionally false results were obtained in this way. Consequently in our recent experiments, including all those reported here, the macroscopic readings have in every case been checked by examination of the sediment in the dark field. The tubes have been incubated for one hour at 37° and allowed to stand over night in the icebox before making readings.

Our preliminary experiments showed that our own strain of *pallidum*, "A," and the strains sent to us by Dr. Noguchi reacted quite similarly in sera obtained by immunizing to strain "A." On testing this serum with other species of treponemata, for cultures of which we are also indebted to Dr. Noguchi, we found there were very strong group reactions. The following is a type of such experiments:

GROUP AGGLUTINATIONS

Serum of Rabbit 624 Immunized with Treponema Pallidum (Strain A)

Treponema pallidum, Strain "A," agglutinated in dilution of	1 : 2000.
Treponema pallidum, Noguchi Strain 1, agglutinated in dilution of	1 : 400.
Treponema pallidum, Noguchi Strain 2, agglutinated in dilution of	1 : 1000.
Treponema calligyrum, agglutinated in dilution of	1 : 4000.
Treponema refringens, agglutinated in dilution of	1 : 200.
Treponema microdentium, agglutinated in dilution of	1 : 1000.

Treponema mucosum, agglutinated spontaneously in the salt solution control.

The spontaneous agglutination of *Treponema mucosum* has made it impossible to obtain satisfactory readings with this organism.

We do not think that too much stress should be laid on the various strengths of reaction with the three strains of *pallidum*, as different suspensions of the same strain tested against the same serum will sometimes show similar differences.

For further information as to the immunological relationship to these organisms, we resorted to absorption tests. The strength of agglutination of all of these organisms in the serum used was first determined as in the following table, and then the concentrated serum mixed with a very thick suspension of the organisms with which it was to be absorbed, usually one part of undiluted serum with nine parts of suspension. After standing for some time the mixture was centrifuged and the supernatant fluid was pipetted off and treated with fresh suspension, and the process was repeated until the supernatant fluid no longer agglutinated the particular organism being used. When the absorption was complete, the serum, which by this time had been diluted from 1 to 25, to 1 to 50 was tested against several different species of treponemata. A type of such experiment is shown in the table on page 18.

As will be seen by the table, the strain of *pallidum* used and also the *Treponema calligyrum* removed all the agglutinins from the serum, whereas the *Treponema refringens* and *microdentium* removed only their respective minor agglutinins. The tonsil organism removed none. The results of other experiments have been quite similar except as to *Treponema refringens*, some suspensions of which have agglutinated very strongly in anti-*pallidum* serum and have absorbed a considerable amount of the major agglutinins. As these results were obtained only occasionally, we are inclined to attribute them to non-specific adsorption by protein particles in the suspension.

The treponema called tonsil organism in these experiments is from a culture which we isolated from an ulcerated tonsil, which is morphologically very similar to the *Treponema micro-*

dentium, but which reacts differently in the agglutinin absorption test.

ABSORPTION OF AGGLUTININS

Preliminary Titration of Serum of Rabbit 323 Immunized with Treponema Pallidum (Strain A)

Suspension of	1 : 10	1 : 50	1 : 100	1 : 200	1 : 500	1 : 1000	1 : 2000	1 : 5000	Salt
Pallidum A	+	+	+	+	+	+	+	0	0
Calligyrum	+	+	+	+	+	+	+	0	0
Refringens	+	+	+	+	0	0	0	0	0
Mucosum	0	0	0	0	0	0	0	0	0
Microdentium	+	+	+	+	+	0	0	0	0
Tonsil organism	+	+	+	+	0	0	0	0	0

Agglutination in Serum of Rabbit 323 (1:50) after Absorption

Serum (1 : 50) Absorbed with:	Tested Against Suspension of:			
	Pallidum	Calligyrum	Refringens	Microdentium
Pallidum A	0	0	0	0
Calligyrum	0	0	0	0
Refringens	+	+	0	+
Mucosum	+	+		
Microdentium	+	+	+	0
Tonsil organism	+	+	+	+
Serum untreated	+	+	+	+
Salt solution	0	0	0	0

We conclude from these observations that the different species of treponemata which we have tested are very closely related, and that the *calligyrum* is indistinguishable from the *pallidum* in these reactions.

We had some hope also that this reaction of agglutination might be of value in diagnosis, and tested first the serum of syphilitic rabbits to see whether it contains more agglutinative substance for the culture pallidum than does normal serum. Normal rabbit serum agglutinates *pallidum* frequently in dilutions of 1 :10, and occasionally shows feeble agglutination in 1 :20 and 1 : 25. Many of our syphilitic rabbits show no stronger agglutination than this, but some show a slight but distinct increase in agglutinative power. The protocol given below is typical of the results obtained in such tests.

AGGLUTINATION OF TREPONEMA PALLIDUM IN RABBIT SERA

Normal Rabbits

	1:10	1:25	1:50	1:100	1:1000
1.	+	0	0	0	
2.	+++	+	0	0	
3.	0	0	0	0	
4.	+++	0	0	0	
5.	+++	+	0	0	

Syphilitic Rabbits

Time since first appearance of syphilitic lesion					
1. 11 days	+++	++	0	0	
2 27 days	+++	0	0	0	
3. 19 days	+++	+	0	0	
4. 2 weeks	++	++	+	0	
5. 11 months	+++	+	0	0	
6. 11 months	++	++	+	0	

Rabbit Immunized with Culture Pallida

1.	+++	+++	+++	+++	+++

The syphilitic rabbit, No. 5, was one that had been twice inoculated; the first lesion developed 11 months ago; the rabbit recovered, was again inoculated, and again developed the disease. The sixth rabbit was one which had a testicular lesion 11 months ago and recently developed keratitis.

The reactions in these animals are slightly stronger than the normal, but are insignificant as compared with the powerful agglutination observed in the rabbit immunized to culture treponemata.

(*c*) TREPONEMACIDAL ANTIBODIES AGAINST TREPONEMA PALLIDUM

After observing the agglutinating effect of the serum of rabbits immunized to cultures of *Treponema pallidum*, we decided to test this serum for treponemacidal activity. The technique of such experiments proved not to be difficult provided that the proper

amount of living treponemata was used for the test. On account of the absence of distinct colony formation, it is impossible to test for any partial killing of the treponemata as is done in the plating method for detecting bactericidal antibodies, and in their case only complete killing or failure to kill completely can be detected. If too small an amount of inoculum is used, successful plants are not regularly obtained, and if too large an amount, the immune serum fails to kill completely.

In preliminary experiments we found that suspensions made up so that 10 to 15 treponemata per darkfield were visible were suitable for the work if 0.1 c.c. of such a suspension was used to inoculate the tubes. The sera tested were those of rabbits and of a sheep immunized against treponema cultures as described in the previous paper, and the sera of normal animals of the same species were used for control. The reactions were set up by distributing the amounts of normal and immune serum to be tested in the high, narrow test tubes used for cultivation of the organism, the total volume in the tube being brought up in each case to 1 c.c. The tubes were then inoculated with an equal amount of broth culture of treponemata and incubated in the waterbath for two hours at 37° C. At the end of this time a small piece of sterile rabbit kidney and a deep layer of ascitic agar covered with liquid petrolatum were added to each tube. The tubes were then incubated for two weeks or until the control showed profuse growth, and then examined microscopically for growth. The tests were set up in duplicate to avoid confusion from a failure of growth due to accidental causes.

The results showed that the normal rabbit serum was treponemacidal when as much as 1.0 c.c. was used, but seldom showed any treponemacidal effect if smaller amounts were employed, whereas immune serum was treponemacidal in amounts as small as 0.01 c.c. and quite possibly in smaller amounts than this, as we did not attempt a quantitative examination of the treponemacidal effect in all cases. The treponemacidal activity of the immune serum was destroyed by heating to 56° C. for half an hour, and such inactivated serum could be reactivated by the addition of 0.05 c.c. normal serum, an amount which in itself showed no

treponemacidal activity. The analogy to the bactericidal antibodies was in this respect complete.

(*d*) AGGLUTINATION OF TREPONEMA PALLIDUM IN NORMAL AND SYPHILITIC HUMAN SERA

The observation of powerful agglutinins in the sera of rabbits immunized to cultures of *Treponema pallidum* led to the hope that similar agglutinins might be demonstrated in the sera of syphilitic patients. It has been recently claimed by Kissmeyer[1] that such agglutinins are found only in syphilitics and that the reaction is of great value in diagnosis, occurring in about 50 per cent. of the cases which give a Wassermann and in some that do not. Our observations on normal human sera showed that such sera frequently agglutinated *pallidum* when mixed undiluted with an equal part of the *pallidum* suspension (dilution of 1–2) and the first experiments were consequently carried out with dilutions of from 1–5 to 1–100.

A preliminary series of tests was made in our laboratory by Miss E. W. Davis. In these tests the diluted serum was mixed with equal parts of a concentrated *pallidum* suspension in narrow agglutination tubes, which were incubated for 1 hour at 37° C., allowed to stand overnight in the ice-box, and the readings then made with the naked eye. We selected for the positive cases in this series tertiary syphilitics with a positive Wassermann reaction, as it was in such cases we felt most hopeful of observing agglutination. The results of these tests (Table I) show a more powerful agglutination in some of the syphilitic sera than in the normal controls.

TABLE I

Macroscopic Agglutinations

	No Agglutination Occurred in	Agglutination Occurred in Serum 1:5 in	Agglutination Occurred in Serum 1:10 in	Agglutination Occurred in Serum 1:20 in
Tertiary syphilis, W. R. +, 23 cases.....	13 cases	2 cases	5 cases	3 cases
Nonsyphilitic diseases, W. R. 0, 5 cases...	5 "	0 "	0 "	0 "
Normal individuals, W. R. 0, 4 cases.....	2 "	1 case	1 case	0 "

[1] *Deutsch. med. Woch.*, 1915, xli, 306.

In observing such reactions microscopically, it was found that the macroscopic readings were occasionally deceptive—that with a treponema suspension, quite free from particles of media, a definite agglutination might be invisible to the naked eye though quite striking under the dark field. On the other hand, it was occasionally found that a flocculation occurred in the tubes in which no agglutination of the treponemata could be observed microscopically. Consequently, in further experiments, all readings were made from tubes set up and incubated as described above, by making a dark field preparation from each tube after disturbing the sediment by drawing it in and out of a capillary pipette. In such preparations, we often met with clumps of a half dozen organisms in the salt controls or in the negative sera, and consequently decided to disregard such slight clumping and consider as positive only specimens which showed large clumps (10 or more organisms) throughout the preparation. Only rarely did we observe the formation of large clumps of innumerable treponemata with complete disappearance of free organisms, an appearance seen in all of our immune sera recorded as ++++. The results of our series of tests carried out in this way are given in Table II. As the work progressed, it was found that so many

TABLE II

Microscopic Agglutinations

	No Agglutination Occurred in	Agglutination Occurred in Serum 1 : 2 in	Agglutination Occurred in Serum 1 : 10 in	Agglutination Occurred in Serum 1 : 20 in	Agglutination Occurred in Serum 1 : 50 in	Agglutination Occurred in Serum 1 : 100 in
Primary syphilis, W. R. +, 6 cases.	3 cases	1 case	2 cases	0 cases	0 cases	0 cases
Secondary syphilis, W. R. +, 18 cases	8 "	4 cases	3 "	1 case	1 case	1 case
Tertiary syphilis, W. R. +, 64 cases	27 "	7 "	23 "	3 cases	2 cases	2 cases
Tertiary syphilis, W. R. 0, 2 cases..	1 case	0 "	0 "	1 case	0 "	0 "
Congenital syphilis, W. R. +, 4 cases.	0 cases	1 case	3 "	0 cases	0 "	0 "
Nonsyphilitic diseases, W. R. 0, 37 cases.....	22 "	4 cases	7 "	1 case	2 "	1 case
Normal individuals, 40 cases.....	35 "	4 "	1 case	0 cases	0 cases	0 cases

nonsyphilitic sera gave agglutination in the 1:2 dilution that observations on sera in this concentration were abandoned after about one half the tests had been done. Consequently, it is probable that a number of the sera recorded as quite negative would have been positive in a 1:2 dilution if tested.

As mentioned above, we decided to disregard the positive agglutination found when undiluted normal serum was mixed with the *pallidum* suspension (1:2) on account of the relative frequency with which such agglutination occurred in the sera of normal individuals. Summarizing Table II, we found that agglutination in dilutions of 1:10 or above was obtained in:

Primary syphilis .....in 2 out of 6 cases, or 33 per cent.
Secondary syphilis .....in 6 out of 18 cases, or 33 per cent.
Tertiary syphilis .....in 31 out of 66 cases, or 47 per cent.
Non-syphilitic diseases .....in 11 out of 37 cases, or 30 per cent.
Normal persons .....in 1 out of 40 cases, or 2 per cent.

As compared with the sera of normal individuals, only one of which agglutinated in the dilutions regarded as significant, positive reactions were frequently observed in the sera of syphilitics. When, however, we compared syphilitic sera with sera of patients suffering from various other diseases and giving negative Wassermann reactions, we found such sera reacted almost as frequently as did the syphilitic. Occasionally also we found very powerful agglutination in cases in which there was absolutely no evidence of syphilis. These findings we are unable to explain. The possibility that the patients had an infection with some other treponema—for example, a throat infection—must be borne in mind. The strong group reactions which we have observed among different species of treponemata would make this seem probable.

The tests in syphilitic sera lead us to believe that, in some cases, especially tertiary cases, there is a small amount of antibody to the treponema developed in the patient's serum. The appearance of this serum immunity is, however, so inconstant and so slight in degree that it seems unlikely that it should have a marked influence on the course of the disease. It certainly indicates that the agglutination reaction is unlikely to be of any value in diagnosis.

(*e*) DIFFERENCES IN SERUM SUSCEPTIBILITY BETWEEN VIRULENT AND CULTURE PALLIDA

Very marked differences were observed between the behavior of virulent treponemata obtained directly from lesions in rabbits and that of treponemata which had been cultivated for a number of generations on artificial media and had completely lost their virulence for animals.

Agglutination experiments showed that the serum of rabbits immunized to cultures, which had a very powerful effect on the avirulent treponemata, agglutinated the virulent organisms very slightly or not at all. Treatment of the virulent organisms by the method of Porges did not render them susceptible to the immune serum. Treatment of virulent suspensions with immune serum before they were injected into animals showed no protective action on the part of the immune serum, although the serum showed in test-tube powerful treponemacidal action against the avirulent organisms. This is more or less in keeping with the fact that infection with the treponemata excites little antibody formation. It is possible that a more definite effect on virulent organisms would be obtained by more powerful immune serum.

(*f*) SPECIFIC COMPLEMENT-FIXATION WITH TREPONEMA PALLIDUM

The writers have utilized a method for the cultivation of *Treponema pallidum* in large quantities for the production of antigen for complement-fixation. For the production of the antigen, slanted egg medium, as for Dorset's egg, is made up in high tubes, covered with ascitic broth, and sealed with paraffine oil. No tissue is needed and the egg can be thoroughly sterilized in the autoclave. The culture treponema grows actively in these tubes so that plentiful growth can be obtained in periods as short as ten days. This enables one to work with young cultures. The material is centrifugalized, ground up with salt, and taken up in distilled water to isotonicity. The writers have paralleled the Wassermann reactions in 159 cases at the time of making this report, with entire identity in 138. There were

21 discrepancies, in a considerable number of which the treponema fixation indicated syphilis which the Wassermann missed. On the other hand, the writers do not regard their reactions, at present, as specific. Experiments indicate that similar antigens can be produced from egg cultures of colon and typhoid bacilli and that a certain amount of the fixation is due to the lipoidal constituents of the microorganism and derived in part from the egg used for cultivation. However, the apparently greater delicacy of this reaction may mean that there is a certain specific element superadded. It may mean, however, that the reaction is more delicate only because a bacterial antigen produced in this way and very delicately titrated (and it is necessary in working with these antigens to approach more closely to the anticomplementary dose) represents simply another way of doing the Wassermann. This, the writers think, is quite probably the case in the many fixations they obtained with the treponema antigen in early syphilis—cases in which their other work does not indicate the existence of antibodies in any great amount.

Further work is being done both in the direction of distinguishing the differences between the treponema antigen and similarly produced antigens from other bacteria, and fixations are being done with the protein and lipoid fractions of the treponema antigen.

Discussion:

Dr. Park: If I understood rightly, it was stated that cultures that had been long grown gave better antibodies than fresh cultures. We have felt that we got better antibodies by injecting our horses with recently isolated cultures. The results reported would throw doubt on this belief.

Dr. Wood: The question of the organism being perfectly adapted to the body so that it does not produce antibodies is a very favorite one with cancer research workers who think that the cancer cell is so perfectly adapted to its environment that the body forms no defence.

Dr. Park: I wonder whether the same amount of killed recently obtained organisms has been compared with the killed cultured *pallida*.

Dr. Ottenberg: I have had an opportunity to watch this work, and to compare the complement fixations with spirochæte antigens in syphilis in parallel tests with the Wassermann reaction, and I cannot help thinking that there must be a specific element in this complement fixation, something more than the physical effect of the antigen, for the reason that the discrepancies come out in a very interesting way. While it was astonishing how regularly

the two reactions agreed, when they disagreed usually the positive reaction with the spirochæte antigen was obtained in a case of tertiary syphilis in which the Wassermann reaction had been positive and had become negative under treatment. The cases in which the Wassermann was positive and the spirochæte test negative were nearly all cases of very early syphilis. If the fixation were due to a physical or chemical reaction with substances other than antibodies in the blood, one would expect it to run parallel with the Wassermann reaction. In a very few cases with no suspicion of syphilis a positive spirochæte fixation was obtained. This may have been a group reaction, such as could perhaps be given if the patient had antibodies for Vincent's spirillum or various spirochætes found in the mouth. The finding of a strong positive Wassermann, with a negative spirochæte reaction in early cases of syphilis, is what one might expect if the spirochæte reaction were due to real antibodies. The Wassermann tests were done by ice-box incubation with alcoholic heart extracts, not cholesterin reinforced—a method we believe to be of greatest delicacy, yet entirely safe. Dr. Swift, in his recent communication to the Academy, expressed the opinion which we have held for some time, that plain alcoholic extracts used in this way act as delicately as the cholesterin reinforced.

Dr. Lapowski: The studies upon spirochætes enlarge our knowledge of the spirochæte; but when a rabbit, inoculated with a pure culture of spirochætes, is referred to as a syphilitic, instead of an inoculated rabbit, the readers assume a far too important rôle for the spirochæte and the rabbit in syphilis. That the spirochæte is *the* cause of syphilis is not as yet proven, and our present knowledge of the relation between the spirochæte and the rabbit rather tends to question the conclusions drawn from such experiments, as to the syphilitic nature of the disease produced in rabbits. The points which have been usually mentioned to prove the specific nature of such inoculated lesions—their histological resemblance to lesions of human syphilis; the positive Wassermann reaction in the rabbits inoculated with spirochæte cultures; the appearance of syphilis-like lesions in places far removed from the place of inoculation—cannot be any more cited in support of the pathogenicity of the spirochæte. The Wassermann reaction is found in normal rabbits and is not considered any more a specific reaction. We must be careful in our estimation of the keratitis parenchymatosa, which occurs in inoculated rabbits, as it has been shown that plain horse serum, injected into a rabbit, produces exactly the same lesions as described under the name of keratitis parenchymatosa, and which are referred to by ophthalmologists as "keratitis anaphylactica." What is of the most importance is the demonstration of the fact that, in 24 per cent. to 30 per cent. of fresh rabbits, taken directly from their farm breeding places, papular ulcerations on the preputium, on the peno-scrotal and anal regions were present and that these lesions contained spirochætes which could not be distinguished from the *pallida*, either by the dark field examination or by Giemsa staining. It is therefore advisable to be careful in identifying the findings in the studies of spirochætes in rabbits with human syphilis, even if the readers of the papers are ready to consider the proposition that rabbits in their normal existence suffer from syphilis.

Dr. Field: Dr. Lapowski has mentioned that 30 per cent. of rabbits have spirochætes. Has anyone demonstrated spirochætes in 30 per cent. of rabbits? The statement also that rabbits have often a positive Wassermann reaction is negatived by most of us who have tested our amboceptor for any positive reaction. We have not found it. In 200 rabbits I have found one which gave a positive reaction. The proposition of finding spirochætes in rabbits and that of calling them *pallida* are two entirely different matters.

Dr. Lapowski: Dr. Field should make himself acquainted with the published reports of other workers in the same field. Regarding the positive Wassermann reaction in normal rabbits I can refer him to the observations of Fleischman, Fischer, Blumenthal and Dohi (*Neisser's Beiträge,* 1911, pp. 515–520) and to the statement of Professor Zinsser in his remarks of this evening. As to the presence of spirochætes which cannot be distinguished from *pallida* in normal rabbits, I can only again refer him to the article of Dr. Arzt (*Wien. kl. Woch.,* 1914, pp. 788–1053) and add that clinically it is often impossible to distinguish in the oral cavity the *Spirochæta dentium* (?) and in the genital organs the *Spirochæta calligyra* from the *pallida.*

Dr. Zinsser: Dr. Park's point about immunization with virulent pallida is extremely important. One year ago we began to immunize with spirochætes from the testicles of infected rabbits, but we did not succeed in getting enough material to get convincing results. We hope in time to get enough material into the rabbit to make the experiment successful. In regard to dead or living organisms—we can deal with living organisms only from cultures. We cannot give the living virulent organisms because we would be likely to get a lesion.

As to Dr. Lapowski's question about the keratitis being syphilitic, as Dr. McBurney has stated, we have not made the claim that it is syphilitic, because we did not succeed in finding spirochætes in the lesion, but we think it is syphilitic because such lesions have been described and proven to be so by other observers. In regard to the spirochætes being *pallida,* I would like to refer Dr. Lapowski to the work of Uhlenhuth and Mulzer. These workers give several points which are strong evidence in this regard. Dr. Noguchi states that he has produced syphilitic lesions with cultivated organisms. Another point is that last year in Germany there occurred in a laboratory a case of syphilis, contracted by a laboratory diener from the fourth generation in rabbits. That to my mind is strong evidence and points to the logical conclusion that the experimental disease in rabbits is true syphilis.

A METHOD OF PRODUCING ANTIGEN FOR COMPLEMENT-FIXATION IN TUBERCULOSIS

H. R. MILLER, M.D., AND HANS ZINSSER, M.D.

(From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University, New York.)

The writers have prepared an antigen for complement-fixation in tuberculosis for which they claim certain advantages over those prepared by previous workers, especially those of Besredka and of Petroff, and the modifications of Besredka's antigen of Bronfenbrenner and Craig. One of the writers (Miller) has especially studied the Petroff antigen in connection with the one reported this evening. There is nothing very original about the method of preparation, which follows closely the endotoxin extraction method previously applied by Besredka to typhoid bacilli and other organisms—namely the grinding up of a weighed amount of bacillary substance with salt, and its subsequent suspension in distilled water until isotonicity is obtained. The antigen so prepared is not anticomplementary in quantities as high as 1.0 c.c. and has given positive fixations with quantities as low as 0.02 c.c. It does not give positive Wassermann reactions with syphilitic sera, that is, it has failed to do so in all but one case out of 26, and tuberculosis could not be absolutely excluded in that case. A total number of 190 cases so far examined are reported. Of these 93 were clinically negative cases the diagnosis being based on careful examination by attending physicians of three institutions, and in all of them the fixation test was negative. In 24 of the 93 positive skin reactions were obtained, but these were adults without clinical signs of tuberculosis. On the other hand 89 cases gave positive reactions, and all of these were actively tuberculous, ranging in degree from the incipient to the very far advanced third stage. This communication is entirely in the form of a preliminary report, but the writers make it because of the regularity of the results in the hope that others will be stimulated immediately to try it.

Discussion:

Dr. Park: If I understood the paper rightly the results were absolutely clear-cut. Cases which were positive clinically gave positive reactions, and those which were negative or too slightly developed to be diagnostic gave no fixation. Thus it seems to me that the test simply corroborated (in most cases at least) what was known already. Apparently there were no doubtful cases in which suspicion was aroused by the reaction. Even this, however, is a great advance and may be perfected to pick up the more doubtful cases.

Dr. Alfred Meyer: I have been interested in the work of Dr. Miller on complement fixation, and I had a number of specimens of blood collected for him. The clinical details of the cases were in my possession, and the conclusions afterwards compared with the serological findings. Among the twenty-six cases one was in the tuberculosis ward at Mt. Sinai. The patient had a history of very severe hæmorrhages. The question arose whether the stomach or the lung was involved. The patient was rather thin, with indefinite lung signs, which I said were not proven tuberculous. There was I thought rather evidence of gravitation of stomach blood giving localized crepitation. The patient was not, however, transferred from my tuberculosis ward. She was kept quiet in bed. She never had fever. The von Pirquet I cannot recall, but she was twenty years old and its result was therefore not important. She had repeated severe hemoptyses while in the ward and the question arose of controlling these with artificial pneumothorax. This was not done immediately, but I said it should be done the next day. I was leaving the ward when one of the house staff came running to me saying that the patient had just had a third hæmorrhage of 20 oz. We then immediately made an artificial pneumothorax and there has been no recurrence of the hæmorrhages for the past eight weeks. A guinea pig injected with the expectorated blood has just been reported "no tuberculosis," and this agrees with Dr. Miller's findings.

This is only one case, but being on the side that Dr. Park criticizes, the one case illustrates the possible value in doubtful cases. This girl is probably not tuberculous, though one must admit that once in a while a positive case of tuberculosis will give no reaction in the guinea pig. The correspondence between the clinical findings and the complement fixation test are to say the least of it striking, even though the results of treatment would justify the view of the pulmonary origin of the blood.

Dr. Kessel: Several of these cases were taken from my service at the Montefiore Home. With two exceptions, all of these patients gave a positive reaction with Dr. Miller's test. The one patient who reacted negatively was a man who had been on the service for many months. He had various attacks of fever lasting from a few days to a few weeks. His sputum was repeatedly negative upon examination. A guinea pig inoculated with his sputum failed to develop tuberculosis. A marked dilatation of the arch of his aorta led us to make the Wassermann test, which was found strongly positive. His response to salvarsan and mercury treatment was a poor one. In view of Dr. Miller's positive findings in the definitely tuberculous patients, the

negative complement fixation result in this case would urge upon us the diagnosis of pulmonary lues.

The other patient who reacted negatively was one who came to us from a tuberculosis sanitarium. He presented evidence at the right apex of a healed lesion, there being dulness and no râles.

In view of the fact that these two cases, one of pulmonary lues, and the other of healed tuberculosis, gave negative results, while all of the other patients examined from the Tuberculosis Service yielded positive results, I believe it fair to regard Dr. Miller's fixation test with considerable optimism.

GAS BACILLUS INFECTION WITH REMARKS ON GASTRIC ULCER

RANDOLPH WEST AND MARY E. STEWART

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Studies were made of a gas bacillus brought from the French front, particularly with regard to its toxin formation, since Weinberg and Sacquépée have recently described a true toxin from an organism belonging to this group. Cultural and morphological examination placed the organism distinctly in the Welch bacillus group. Filtrates of anaerobic broth cultures killed guinea pigs but it was found that this action could not be attributed to a true toxin, since neutralization of the acid filtrate removed this toxicity. Suspicion that the toxic effect was due to the acidity of the filtrates led to the injection intraperitoneally and intravenously of acid solutions equivalent in acidity to the toxic filtrates of the cultures. The gas-bacillus infected guinea pigs, in the large majority of the experiments, showed gastric ulcers. Gastric ulcers were also noticed in the guinea pigs injected intraperitoneally and intravenously with acid. Gastric ulcer, without death of the animal, occurred regularly after intravenous injection of 4 to 4.5 c.c. of 1 per cent. acetic acid. The acid solutions were not as uniformly toxic as the acid filtrates, possibly because of the presence in the acid filtrate of butyric acid which is highly poisonous.

Discussion:

Dr. Libman: This paper is of great interest to me because in 1901 I drew attention to the probable importance in the production of the phenomena of disease of the acid produced by bacteria when grown in carbohydrate medium. At that time and later I noted a number of features of growth of bacteria in the presence of carbohydrate and serum that had been overlooked. The important facts are the following: Precipitation of proteid may occur. Pigment and smell production may be curtailed or inhibited. In media containing blood and carbohydrate, agglutination was sometimes produced and at times coagula.

One must believe that these peculiarities of growth in the presence of carbohydrate must be significant as regards infection in diabetics in whom there is usually 0.5 per cent. glucose in the blood. They may even be of importance in non-diabetics in whom the proportion of glucose in the blood is about 0.1 per cent. and in whom until a part is closed off from the general circulation there will be a new supply of carbohydrate.

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PATHOLOGICAL CHANGES IN THE LIVER IN CHRONIC PASSIVE CONGESTION[1]

R. A. LAMBERT, M.D., AND B. R. ALLISON, M.D.

(*From the Pathological Laboratory of the Presbyterian Hospital, New York*)

It is well recognized that the liver in some cases of extreme chronic passive congestion shows a more or less extensive central necrosis, while in other instances a great dilatation of the capil-

[1] A more detailed paper on this subject will appear in an early number of the *Johns Hopkins Hospital Bulletin.*

laries without necrosis is found. In many of the older textbooks, and in certain of the recent ones, the second type of lesion has received the greater emphasis. The disappearance of the liver cells about the central hepatic veins is attributed by most authors to a progressive atrophy from increased blood pressure in the dilated capillaries.

Mallory, among others, has objected to this interpretation of the lesion, maintaining that the effect of increased blood pressure would be felt equally by all of the cells in the liver lobule. He regards the disappearance of the cells in the central portion of the lobule as always the result of a toxic necrosis associated in most cases with hemorrhage. The toxic factor, he suggests, is a terminal bacterial infection, so commonly observed in persons dying from chronic disease. He has supported this view by demonstrating the frequent occurrence of central necrosis or degeneration of liver cells, in a large percentage of cases of acute infection not associated with chronic circulatory disturbance.

The chief object of the present study was to determine, first, whether the two lesions referred to, which may be conveniently designated "dilatation atrophy," and "hemorrhagic necrosis," exist independently, and, secondly, to discover the factors that determine the presence of each.

The material for this study was selected from 500 autopsies at the Presbyterian Hospital, among which there were about 90 cases of well-marked chronic passive congestion.

It may be stated in the beginning that a careful study of the livers of these cases has convinced us that there are not *two*, but at least *five* types of hepatic lesion associated with chronic passive congestion. While it must be admitted that this division into so many groups is in some respects arbitrary, and that many livers present more than one type of lesion, we believe that the types described are sufficiently well defined for practical consideration.

The general characteristics of the five types, together with the form of cardiac or circulatory disorder with which they are most often associated, are given in the following table:

Classification of Lesions

Type	Character of Lesion	Diseases with which Lesion is Most Often Associated	Number of Cases	Per Cent.
I	Capillary dilatation, central or diffuse, with atrophy of liver cells. No necrosis.	Arteriosclerosis, myocardial fibrosis, chronic nephritis with cardiac failure, moderate cardiac valvular disease.	32	31
II	Capillary dilatation (often not marked) with central degeneration of liver cells. No hemorrhage or definite necrosis.	Same as type I.	17	16.5
III	Marked central fat accumulation with mid-zonal capillary dilatation. Areas of hemorrhage and necrosis (type IV) often present.	Cardiac valvular disease, especially chronic rheumatic forms in young individuals.	9	9
IV	Central necrosis with hemorrhage.	Advanced cardiac valvular disease (especially cases with involvement of several valves), extreme myocardial fibrosis, arterio-venous aneurysm.	28	27
V	Central "collapse fibrosis." Lesion is usually secondary to type IV.	Prolonged circulatory disorder of any kind.	17	16.5

The separate grouping for the cases included in type III (central fat accumulation and mid-zonal capillary dilatation) may not appear warranted since an accumulation of fat is observed in many of the cases belonging to other types (especially II and IV). The gross and microscopic picture, however, is so strikingly different that we have felt justified in setting them apart. This particular type of lesion has been very fully described by several German authors.

While the relationship of these several types of lesion is not altogether clear, histological studies have, to a certain extent, made clear the sequence of changes. There can be little doubt, for example, that the "collapse fibrosis" (type V) is secondary to the changes described in type I or type IV, the disappearance of the liver cells taking place in the one case by a process of atrophy associated with capillary dilatation, and in the other by a more sudden necrosis with hemorrhage. We have studied several livers

which showed very clearly transitions from type IV to type V. In the "collapse fibrosis" following hemorrhagic necrosis an enormous quantity of hematogenous pigment lying free or phagocyted by surviving endothelial cells is present. We have furthermore observed many instances of transition from type I to type II and type IV, also from type III to type IV.

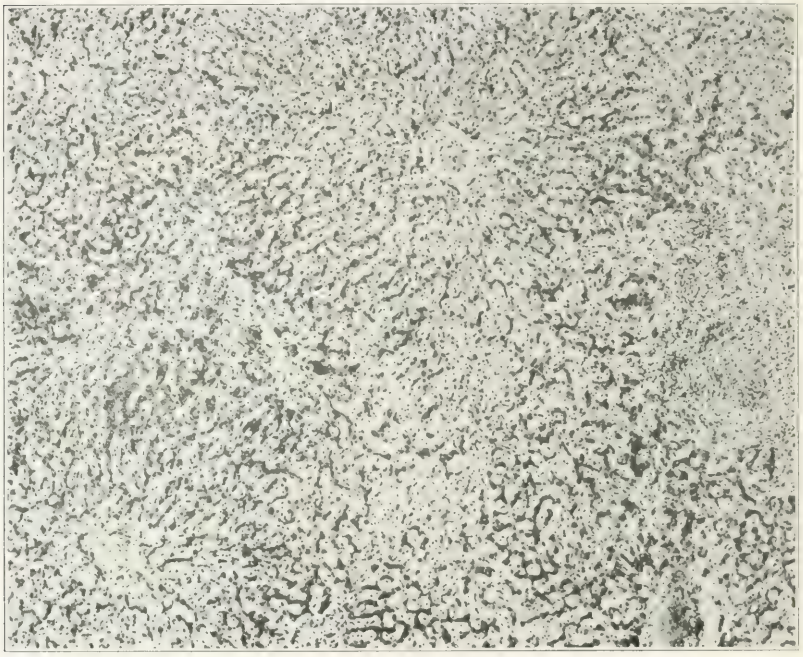

FIG. 1. Chronic passive congestion of liver, type I, showing marked dilatation of capillaries with atrophy of liver cells. Case of arteriosclerosis, and chronic pulmonary tuberculosis, with a terminal generalized miliary tuberculosis.

It is clear therefore that the liver may pass through several stages of chronic passive congestion represented by at least three of the types mentioned above, ending in type IV or type V. It is equally clear, however, from histological studies, that in some cases a rapid and extensive necrosis has taken place, not preceded by any marked dilatation of the capillaries, since at the margin of

the necrotic zone, which is often sharply defined, no dilatation of the capillaries whatever is seen.

In an effort to clear up the question as to why at autopsy one liver should show only capillary dilatation and atrophy of the central cells (type I) while in another case extensive central ne-

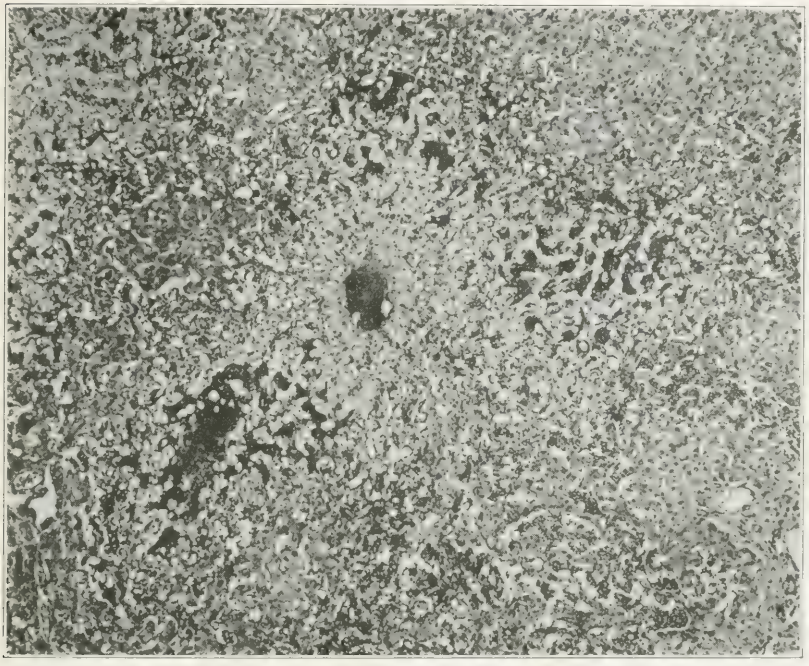

FIG. 2. Chronic passive congestion of liver, type III, showing marked central fat accumulation with mid-zonal capillary dilatation. Case of chronic rheumatic endocarditis, involving mitral, aortic and tricuspid valves; acute pericarditis and pleurisy.

crosis is seen, we have examined the clinical histories and autopsy protocols of the cases and tabulated them with reference to the following points: (1) The existence of a terminal complicating infection and (2) the severity of the lesion in the heart or vascular system, responsible for the circulatory disorder, for example, the degree of valvular stenosis or myocardial change, number of valves involved, etc.

An examination of this table has shown, first, that terminal infections were as common in cases belonging to type I, in which there was no necrosis, as in those of type IV, showing hemorrhagic necrosis. Cases in which there was a definite septicæmia were somewhat more common in type II (capillary dilatation with central degeneration).

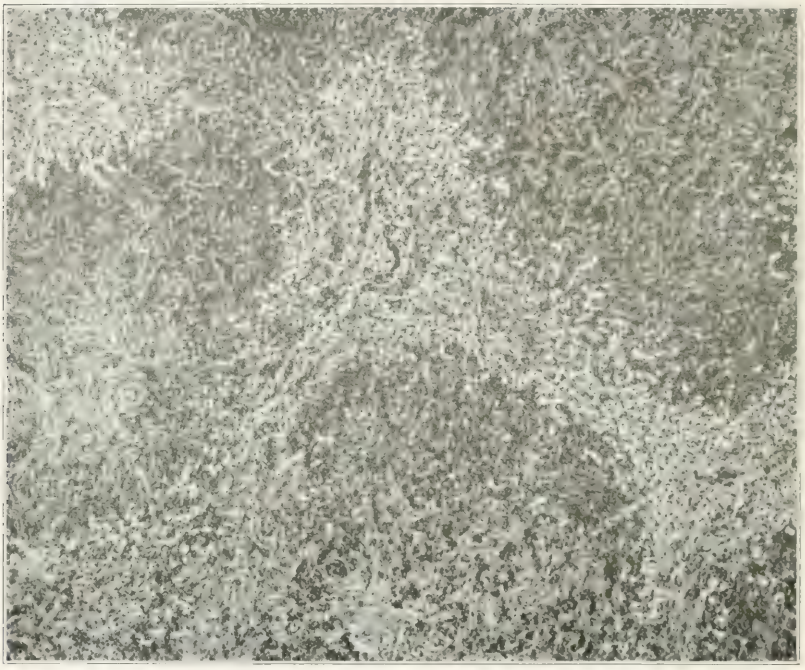

FIG. 3. Chronic passive congestion of liver, type IV, showing extensive central necrosis with hemorrhage and fatty infiltration of surviving peripheral cells. Case of myocardial fibrosis, generalized arteriosclerosis, adherent pericardium.

It was found, secondly, that in all of the cases with extensive central necrosis and hemorrhage (type IV), there was evidence clinically of extreme circulatory disorder, and that the anatomical changes in the heart or vascular system responsible for the chronic congestion, were very marked. For example, of the five cases selected as showing the most extreme necrosis, three were ex-

amples of chronic cardiac valvular disease with involvement of mitral, aortic and triscuspid valves; the fourth was a case of extreme mitral stenosis; in the fifth, where the most extensive lesion of all was present, the cause of the circulatory disturbance was an aneurysm of the arch of the aorta which had ruptured into the superior vena cava.

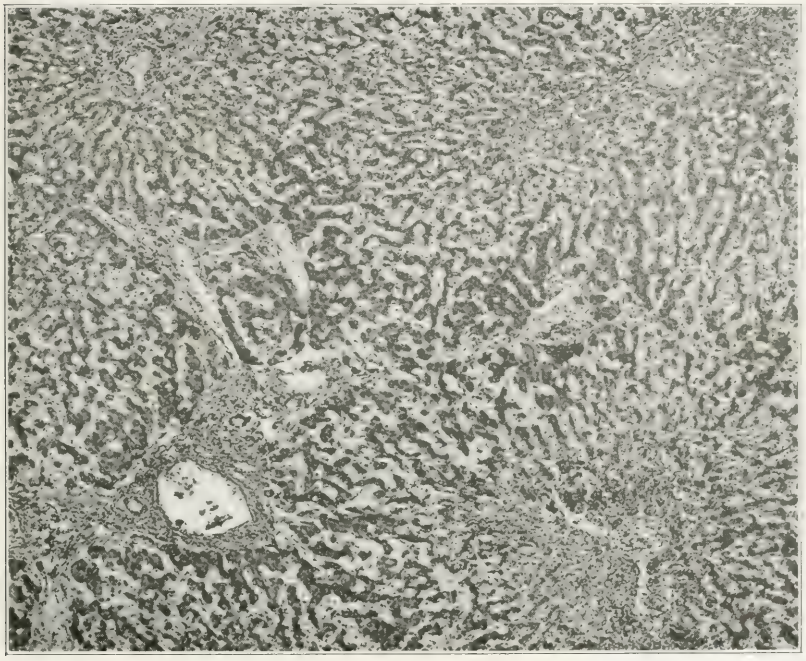

FIG. 4. Chronic passive congestion of liver, type V, showing central "collapse fibrosis." Case of aortic stenosis.

These findings would indicate therefore that the factor most responsible for the necrosis of the liver cells is the degree of circulatory disorder, and that terminal infections are of little or no etiological importance.

Discussion:

DR. PAPPENHEIMER: It is very striking to see how abruptly the necrosis stops in the hemorrhagic areas. I would like to know if Dr. Lambert has found any explanation that will throw light on this. On one side of the line there are perfectly normal liver cells; on the other there is just a mass of blood. The division is very sharp.

Dr. Wood: The more advanced lesions, especially the hemorrhagic, suggest a bacterial infection. Some of the experimental toxic liver lesions show extensive hemorrhage without necrosis; some show extensive necrosis without hemorrhage. It seems to me that the walls of the capillaries and sinusoids must be injured by some sort of poison to permit the blood to escape.

Dr. Lambert: Answering Dr. Pappenheimer's question, I think that the sharp line of demarcation between healthy and necrotic cells in cases of extreme chronic passive congestion, must in some way be referable to the peculiarities of the circulation in the liver with its two blood supplies under different pressures, meeting in the mid zone of the lobule. But I am unable to explain the exact mechanism of the process.

Regarding Dr. Wood's remarks, I do not believe it necessary to assume the existence of a toxin acting on blood vessel walls in order to explain the hemorrhagic character of the lesion. My idea is that a condition of marked stasis exists as the result of which both liver cells and endothelial cells suffer. After the disintegration of the liver cells, the blood of the engorged capillaries pours through the injured walls into the trabecular spaces. In other words, while the hemorrhage does result from an injury to the vessel wall, this injury is due simply to malnutrition or asphyxia of the cells as in hemorrhagic infarction.

A CONVENIENT STAIN FOR CONNECTIVE TISSUE

S. LOWENTHAL

(*From Columbia University, George Crocker Special Research Fund, F. C. Wood, Director*)

In the staining of tissues great difficulty has been encountered in the attempt to bring out distinctly the fine connective tissue structures without obscuring the other elements. Of the various connective-tissue stains at our disposal those of Mallory and of Van Gieson are the ones most commonly used. A permanent and a very satisfactory connective-tissue stain is that of Maresch-Bielchowsky, but as the process is long and demands the undivided attention of the experimenter it is not used as a routine stain. Although Mallory's stain often gives satisfactory results after Zenker's fixation, it does not act uniformly, and owing to deterioration, a fresh solution of the stain must be made up frequently. Both Mallory's and Van Gieson's stains are apt to fade in time unless very carefully mounted.

The following method gives excellent results as a differential

and connective-tissue stain and seems quite permanent, stained sections having retained their color for over two years. The process is very simple, and the results are uniform. Zenker's fixation is the only requirement.

Method: Tissues fixed in Zenker's are embedded in paraffin. After thin sections have been cut, they are fastened to slides and allowed to dry. The paraffin is removed, the sections are treated with iodine to remove the mercury, and with hyposulphite of soda to remove the iodine, and then washed thoroughly in several changes of distilled water.

Two solutions are to be kept in stock:

I. A saturated aqueous solution of basic fuchsin (3.5 gm. to 100 c.c. distilled water).

II. Aniline blue (Grübler) ..... 0.5 gm.
Saturated aqueous solution of picric acid ..... 100 c.c.

The staining process is as follows:

1. Place the sections in solution I for 5 minutes;
2. Wash off excess of stain in distilled water;
3. Transfer to solution II for from 15–30 seconds;
4. Dip in distilled water;
5. Dehydrate in 95 per cent. alcohol until no bluish color is given off;
6. Dip in absolute alcohol for a few seconds;
7. Clear in xylol and mount in acid free gum dammar.

Results: The connective tissue is stained a bright blue. The parenchymal cells of the organs take a green color, with the exception of mucous and preputial gland cells. The cells of the mucous glands take a pink stain in contrast to the green or bluish-green color of the serous glands. The preputial gland cells are stained yellow, and the cells of the sebaceous glands light blue. Nuclei, extremely cornified epithelium, and the intercellular substance of old hyaline cartilage, are stained red. Red blood corpuscles are stained yellow; striated and smooth muscle, epithelium, and nerve tissue are stained green.

This stain can also be used advantageously after formalin fixation. Sections which have been fixed in formalin are fastened

to slides and are allowed to dry. The paraffin is removed and the sections are brought down to water. They are then placed in Zenker's fluid over night; the Zenker's fluid may be used with or without the acetic acid. The sections are then washed in water and treated as described above for the ordinary Zenker's fixation.

As far as can be ascertained there is no record of any other composite stain of this nature showing the same results, except that of Borrel; but Borrel's stain is difficult to use; it does not give uniform results; and it fades in some cases.

PRESENTATION OF SPECIMENS

A. B. EISENBREY, M.D.

(*a*) An Amyloid Tumor of the Larynx

The first case that I wish to report is one of an amyloid tumor on the left vocal cord of a woman sixty-six years of age, a patient of Dr. Voislawsky at St. Luke's Hospital. It was regarded as a neoplasm, possibly malignant, and was excised. We found that there was no evidence of actual neoplasm at all. The laryngeal mucosa was intact, but the underlying structures were heavily infiltrated with amyloid material. This was of more or less homogeneous structure but showed in some areas a lamellar arrangement. It was seen in the lymph spaces and loose in the tissues. Some of the masses showed a structure similar to prostatic corpora amylacea.

Few cases of amyloid tumor of the larynx have been described. Kaufmann alludes to three. Infiltrating and nodular types are distinguished. Amyloid degeneration in sarcoma and other neoplasms is very rare.

(*b*) Two Types of Carcinoma Occurring in the Same Uterus

The second case is one of adenocarcinoma and epithelioma occurring in the same uterus. The patient was a widow fifty-

nine years old, on the service of Dr. Mathews at St. Luke's Hospital. A hysterectomy was performed and the first specimen examined was diagnosed epithelioma. It represented a limited area but showed what appeared to be a small portion of adenomatous growth. In sections from higher up in the uterus an apparently separate and distinct adenocarcinoma was found. Sections through the entire posterior wall of the uterus show an epithelioma in the region of the cervix, and an adenocarcinoma in the fundus. The majority of cases which have been described as showing two types have been discarded on the ground that the portions described as epithelioma were merely areas of metaplasia in an adenocarcinoma which had the general appearance of squamous epithelium, on account of metaplastic changes in the glandular cells. I believe that there are two distinct growths in this case.

Discussion:

Dr. Pappenheimer: I would like to ask whether the amyloid in these localized amyloid tumors is usually found in the lymph spaces.

Dr. Eisenbrey: Yes, in the lymph vessels and in the walls of the blood vessels, and also, in the larger nodular form, in the tissue clefts.

Dr. Lambert: Do these amyloid growths give all the characteristic reactions of amyloid?

Dr. Eisenbrey: Sections of this tumor react to gentian violet.

Dr. Lambert: Do they react to iodine? Iodine and sulphuric acid reactions are obtained only in the later stages of amyloid change.

Dr. Wood: They did not give the iodine reaction.

Dr. Mandlebaum: In reference to multiple primary tumors of the uterus, one must be very guarded in establishing the diagnosis. The cases in which most uncertainty may exist are those which seem to show a squamous cell carcinoma of the body of the uterus, associated with adenocarcinoma of the cervix; for metastases from the cervix may occur, and if sufficient metaplasia is present, the secondary tumors may appear to be primary growths of squamous type situated in the body of the uterus. About ten years ago Hitschmann analyzed the cases that had been reported as primary multiple tumors of the uterus and seemed to prove that all of the reported cases were single tumors with metaplastic changes. In the case presented by Dr. Eisenbrey this objection can hardly be raised, for here the squamous type is in the cervix and the adenomatous type in the body of the uterus.

In looking up the literature some years ago I found that Klien in 1894 reported the first case of two tumors localized in the cervix, a squamous cell carcinoma of the portio, and a cylindrical cell carcinoma arising from an erosion in the cervical canal. This was probably the only case of multiple

tumors of the uterus that Hitschmann could not dispute. In 1907 I had the opportunity of studying a similar case, which is, very likely, the second one of this kind on record. The specimen was obtained from a woman of fifty-one years with a large necrotic mass occupying the site of the cervix. Some of the sections showed pure adenocarcinoma, others squamous cell carcinoma, and, as a rule, both types were sharply separated.

In tumors of this kind situated in the cervix, three possibilities of interpretation must be considered. (1) The tumor may be unicentric in origin, arising from cylindrical epithelium and showing distinct metaplasia. (2) The tumors may be pleuricentric and spring from two separate anlages. (3) The tumor may arise from a single center of transitional epithelium where the flat cells pass over to the cylindrical type, and both types of tumor be produced. Even though two distinct types of tumor were present in my case, I hesitated to report it at the time, because a limited number of the cylindrical cells showed definite metaplasia. Dr. Cullen examined the slides and was inclined to think that the entire tumor was adenocarcinoma with metaplasia. Dr. Welch, on the other hand, agreed that the cervix was the seat of two distinct tumors.

Dr. Lewisohn: When I saw the announcement of Dr. Eisenbrey's presentation, I recalled to my mind a paper on metaplasia which I published about ten years ago. I reported two cases of metaplasia: (1) Cancroid of the pancreas, and (2) psammocarcinoma of the uterus. Both of these cases showed typical metaplasia. The transformation from cylindrical to squamous cell epithelium is very well demonstrated in the pictures which I pass around. There can be no doubt that in these cases we are dealing with true metaplasia. In many instances of two different types of carcinoma found in the same organ, the question whether the cause is a true metaplasia, or whether the apparent metaplasia originates from embryonal rests, is open to discussion. In my case of cancroid of the pancreas the latter possibility is out of the question, as this organ is in all its embryonal stages far removed from any ectodermal epithelium. In cases of double carcinoma of the gall-bladder a chronic irritation caused by stones has often been assumed to be the cause of the metaplasia. This is entirely out of the question in the pancreas case, as no stones were found. I would like to ask Dr. Eisenbrey if the tumors were very far apart, whether there was any possibility of a change in character of the cells from one kind to the other, or if such a possibility did not exist.

Dr. Eisenbrey: The areas were perfectly distinct in this case. The two types of tumor also were quite different. The adenoma was well up in the fundus; the epithelioma in the cervix. There were no cells of the squamous type to be seen in the area of the adenocarcinoma.

Dr. Wood: It seems to me from my study of the specimen that the two growths are distinct. I have seen many instances of metaplasia; apparently it is of frequent occurrence in old women. There is also squamous-cell hyperplasia—a sort of leucoplakia of the uterus. In this case there seems to be no point of contact between the two tumors and the difference in type is preserved. I have seen carcinomata of two types in the same uterus which metastasized and in the metastases preserved the two types.

DR. MANDLEBAUM: In the study of a large series of tumors of the bladder I found five cases, mostly in elderly subjects, of typical squamous-cell carcinoma. A chronic inflammation such as cystitis, or a long-continued irritation due to the presence of a calculus, may result in an area of flat epithelium from metaplasia. If a carcinoma arises from this site it will naturally be squamous cell in type.

DR. WOOD: The case of the laryngeal tumor is the first that I have seen. It is necessary to be rather cautious in making a diagnosis of amyloid tumor in tissues about the larynx, as some fragments of thyroid gland, the colloid of which may be mistaken for amyloid, are not infrequently distributed in the tissues surrounding the gland or even at a considerable distance from it.

A SUPRARENAL ADENOMA IN THE MOUSE

SHIGEMITSU ITAMI, M.D.

(From Columbia University, George Crocker Special Research Fund, F. C. Wood, Director)

A search of the literature reveals only a few cases of hypernephroma in the mouse (Tyzzer, Haaland), all of which were situated in the kidney; the present example, so far as is known, is the only one in which such a tumor has involved the adrenal itself.

On June 28, 1915, a brindle female mouse (440/0), weighing 26.5 grams, was received at the laboratory; it appeared to be old, though its exact age could not be ascertained. A tumor in the left axilla, $2 \times 1 \times 1$ cm., which proved to be an adenocarcinoma, was extirpated one week later. The mouse died, without recurrence, on November 12.

At autopsy, the left adrenal gland was found enlarged to about half the size of the kidney, and surrounded by an extensive hemorrhage; both kidneys and ureters, as well as the right suprarenal, were normal. The enlarged adrenal with its corresponding kidney was fixed in Zenker's fluid. As the amount of material was very small, no attempt at transplantation was made.

Microscopic examination of serial sections showed that the portion of the capsule facing toward the kidney was torn and hemorrhagic. The cortex was stretched, and compressed against

the capsule, by a growth occupying the central region of the gland and appearing to originate in the medulla.

The tumor was clearly defined from the cortex, which it showed no tendency to invade. It was composed of solid alveoli, separated from one another by delicate connective-tissue strands and thin-walled capillaries. The individual cells were of moderate size and polyhedral shape, possessing rather small, vesicular nuclei and a well-stained protoplasm. No mitotic figures could be found. Though the cells resembled much more closely those of the medulla than those of the cortex, it is difficult to draw an exact conclusion regarding the site at which the tumor arose, though it probably sprang from the medulla. The resemblance to human tumors of similar origin was very close.

The comparatively regular arrangement of the cells composing the neoplasm, their fairly uniform shape and size, the expansive growth, and the absence of mitotic figures, all combine to suggest a diagnosis of adenoma.

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Discussion:

Dr. Pappenheimer: Did I understand the doctor to say that the cells were probably medullary. The Zenker gives a reaction with chromaffin tissue.

Dr. Itami: The tumor looks like human adenomata in the adrenal; some of these arise from the cortical tissues, others from the medulla. We were not able to get a chromaffin reaction.

Dr. Pappenheimer: Is it not rare to find a tumor containing chromaffin tissue arising only from the medulla? I think this is exceptional.

A CASE OF SENSITIZATION TO WITTE'S PEPTONE

K. R. COLLINS, M.D.

The following case may be of interest on account of a very definite sensitization to peptone developed in a laboratory worker. I am not aware of such a case having been reported before. The facts are as follows:

The patient, Mr. M., twenty-five years old, in apparent good health, was employed in the laboratory of the Georgia State Board of Health from September, 1913, to September, 1915. Previous to this period he served in the army, and in April, 1911, was given typhoid vaccine. In June, 1914, he decided to take typhoid vaccine again. One half dose was given to him. This vaccine was grown in broth. Within a few minutes after the administration of the dose the patient complained of a choking sensation and became markedly cyanotic. The condition passed off in a few minutes, but on account of the severity of these symptoms subsequent inoculations were omitted. Five months after this second attempted immunization to the typhoid bacillus the patient complained of an irritable throat, and upon examination the Vincent's bacillus was found to be present. About this time he found that he was sensitive to peptone. A few minutes after handling peptone he would complain of constriction of the throat, and then develop a coryza and conjunctivitis, and a marked rash upon the face, principally about the eyes. The eyelids became swollen and edematous. On one occasion after weighing out peptone the attack was extremely severe, and the symptoms of collapse were so marked that strychnin was administered. He was then instructed to refrain from handling peptone in any way, but it was found that he could not even go into a room where peptone was being weighed or where a bottle of peptone was open. All peptone was then handled in one department, and he was instructed to keep away from this department. It was found also that the inhalation of the fumes of boiling broth after the peptone had been added developed this same condition, though the preparation of the broth up to this point did not affect him. He remained free from these attacks as long as he was not exposed to peptone. Witte's peptone only has been used in this department, and in all other departments of the laboratory until quite recently. I do not know whether he was exposed or not to the American peptone which is now being used in some cases.

On two different occasions, about one hour after the attack began, his nose, throat and eyes were examined by Dr. A. W. Stirling, oculist, who gives the following report: April 25, 1915,

Pharynx—slightly granular. May 11, Eyes (right)—chemotic conjunctiva; (left)—same to a less degree. Nose—slight serous discharge. (Had used a little chloroform.) Glottis—evidently was edematous from the description of symptoms, but no evidence of this at time of examination.

Attacks were not brought on by inhalation of the dust from hay or of any other substances than the peptone. A part of his duties was the inoculation and bleeding of horses and the handling of guinea pigs, and he remained unaffected while doing this work.

Later he began to run an afternoon temperature and tubercle bacilli were found in his sputum. He was then advised to give up the laboratory work, which he did in September, 1915. His temperature has since become normal and the tubercle bacilli have apparently disappeared from the sputum, but a large number of Vincent's bacilli remain. My object in mentioning the Vincent's bacillus is that as we have had quite a number of sore throats, apparently due to this organism, it is possible that the first irritation of the mucous membranes of the nose and throat permitted the absorption of peptone.

I would like to call attention to the fact that Mr. M. had been in the laboratory and had handled peptone a year before he gave any signs of sensitization to this substance.

Discussion:

Dr. Eisenbrey: I know of a dean of a medical school who is sensitive to rabbits. He has an anaphylactic attack if he goes near these animals.

Dr. Wood: I have seen a man who is sensitive to mice, and for this reason was compelled to leave a laboratory in which he was employed.

Dr. Hopkins: This is apparently an instance of acquired sensitization to peptone, though I do not believe it is possible experimentally to sensitize animals to albumoses or peptones. The extreme sensitiveness of this patient resembles more the natural sensitiveness that Dr. Longcope is inclined to classify separately from the experimentally or spontaneously acquired type. The fact that the patient was not susceptible to peptone when first observed would, however, exclude him from this group.

A MODIFICATION OF RÖMER'S INTRACUTANEOUS METHOD FOR THE DETERMINATION OF SMALL AMOUNTS OF DIPHTHERIA ANTITOXIN IN BLOOD SERA

ABRAHAM ZINGHER, M.D.

(From the Research Laboratories of the Department of Health, New York City)

A convenient, practical and economical test to determine the diphtheria antitoxin content of blood sera is important, in that it would help to give us a solid scientific foundation in many experimental and clinical problems connected with the subject of diphtheria. The older subcutaneous test of Ehrlich for determining the antitoxin content by the death of the guinea pig has been universally accepted as a standard, but it cannot be used with any degree of accuracy, where less than 1/20 of a unit of antitoxin per c.c. of serum is to be determined. A method, therefore, that would be accurate, and enable us to determine as little as 1/200 unit of antitoxin, require but a small amount of serum, and be saving in animals, would be of considerable advantage.

In 1909 Römer and Sames[2] published a method for estimating small amounts of antitoxin in bloodsera. They determined the necrotizing dose of a toxin, *i. e.*, the amount of toxin which added to one unit, or a fraction of a unit, of antitoxin, was still capable of producing a slight local necrosis in the guinea pig. This was called the Ln dose of the toxin. Römer and Sames thus found that 1/500 M. L.D. of the toxin was sufficiently neutralized by 1/40,000 unit of antitoxin to just produce a slight local effect in the animal. This amount of antitoxin in 1/20 c.c. of undiluted serum (the quantity used in the test with an equal volume of toxin solution) represents 1/2000 unit of antitoxin per c.c.

The method suggested by Römer and Sames has not given reliable results in the hands of some other workers who have tried it repeatedly. The difficulties lie, first, in their attempt to make

too fine a distinction between the various lesions of close lying tests. They thus claim to be able to distinguish a 5 per cent. difference in antitoxin content between the individual tests. A second point is the long time, twenty-four hours, required for the toxin and serum to stand before injection into the animal. Thirty minutes has proven to be a sufficient time to allow for the full union between toxin and antitoxin, even in the high dilutions used in the test. A third factor lies in the necessity of establishing a new toxin test-dose, the Ln. These factors have undoubtedly discouraged the use of a test which is inherently valuable.

The modification of Römer's method which I shall describe has been found simple and workable in testing many hundreds of human and animal sera. The L+ dose of toxin is taken as the standard and the toxin is so diluted that each cubic centimeter represents 1/100 L+ dose. A small amount of this dilution is mixed with an equal amount of whole serum, or of serum diluted with salt solution, and 0.2 c.c. of the mixture is injected intracutaneously into a guinea pig. In a series of such tests, made with graded dilutions of a given serum, two points are determined: the test containing the smallest amount of serum at which no lesion develops, and the test containing the largest amount of serum at which a distinct lesion appears. Within these two limits lies the antitoxin content of the serum. If the series changes abruptly from no necrosis to marked necrosis, an intermediate test is made. For example, if there is slight necrosis at "one unit" (toxin 1 : 100 + serum 1 : 100) and no necrosis at "one half unit" (toxin 1 : 100 + serum 1 : 50), the antitoxin content is one unit. If necrosis is marked the test is repeated for three quarters of a unit.

The method presents a number of advantages. It saves animals in that four tests can be made on each guinea pig. It also saves time in that the tests can be interpreted in 48 to 72 hours. As little as 1/200 unit of antitoxin can be determined in a serum with a fair degree of accuracy. If a distinct lesion appears at this test the serum is considered as having no antitoxin.

The details of the test are as follows:

1. The test-toxin. A standard, well-ripened toxin is used, so diluted with normal saline that 1 c.c. represents 1/100 L+ dose. A fresh dilution is prepared for each set of tests, as shown in the accompanying table.

2. The serum to be tested is used either undiluted or diluted 1 : 10 (0.2 c.c. serum + 1.8 c.c. saline), 1 : 100 (0.2 c.c. of serum dilution 1 : 10 + 1.8 c.c. saline), etc. The volume of serum used in the test is always 0.2 c.c., except where the test is made for 1/200 unit of antitoxin, when 0.4 c.c. of undiluted serum are added to 0.2 c.c. of toxin solution.

3. Normal salt solution is added to balance the mixture.

4. After the addition of toxin, serum and salt solution, the mixture is allowed to stand for 30 minutes at room temperature before injecting it into the guinea pig.

5. The guinea pigs (300–350 gm.) are prepared by removing the hair from the abdomen. This is best accomplished by simply pulling it out, leaving a central line of hair to divide the abdominal surface into two halves.

6. An animal holder is quite essential for accurate work and facilitates the exact placing of the injections.

7. A 1 c.c. "Record" or tuberculin syringe and a fine steel or platinum-iridium needle are necessary.

8. Four injections are made into each guinea pig, the abdomen being for this purpose divided into a right upper (R.U.), right lower (R.L.), left upper (L.U.) and left lower (L.L.) quadrants. The injections are made as far apart as possible to avoid a fusion of the lesions. Two tenths c.c. of each test-mixture is injected intracutaneously.

9. The places of injection on the guinea pigs are examined at the end of 24, 48, 72 and 96 hours and a careful record is kept of each test. The following changes in the local appearance of the skin at the site of injection are noted:

(*a*) *Redness* whether marked or slight.

(*b*) *Induration* whether marked, moderate or slight.

(*c*) *Necrosis*, marked or slight or only superficial scaling. The induration is most marked at the end of 48 or 72 hours, the necrosis begins to appear at the end of 72 hours.

Intracutaneous Estimation of Diphtheria Antitoxin

This table shows the amount of toxin solution used, the dilution of the serum, and the quantity of normal saline added to balance the mixture. The figures in the right hand columns indicate the number of units of antitoxin per c.c., in a serum which prevents the production of more than a slight necrosis when mixed with toxin in the proportions indicated.

Test Toxin 1 c.c. = $\frac{1}{100}$ L+	Normal Saline	Serum				
		Undiluted 0.2 c.c.	1 : 10 0.2 c.c.	1 : 100 0.2 c.c.	1 : 1000 0.2 c.c.	1 : 10,000 0.2 c.c.
0.2 c.c.	0.0 c.c.	0.01 unit	0.1 unit	1 unit	10 units	100 units
0.4 c.c.	0.2 c.c.	0.02 "	0.2 "	2 units	20 "	200 "
1.0 c.c.	0.8 c.c.	0.05 "	0.5 "	5 "	50 "	500 "
2.0 c.c.	1.8 c.c.	0.1 "	1.0 "	10 "	100 "	1,000 "

CONCLUSIONS

1. An intracutaneous method is presented for estimating, in an economical way, small amounts of antitoxin.

2. The test is easily carried out. It requires for accuracy, however, a uniform technique in making the dilutions of toxin and serum, the allowance of a uniform time for the two to combine, uniformity in the method of injection into the guinea pig, daily observations of the animals, and uniform interpretation of the tests.

3. This test is of value in experimental work in diphtheria, and in the accurate serological control of actively immunized individuals.

Discussion:

Dr. Hopkins: I should like to ask about the quantitative relation between this and the subcutaneous test. Is the L+ dose the same as the Ln?

Dr. Zingher: The two tests do not exactly correspond. The L+ dose of toxin is very convenient for use. It represents a definite amount of toxin, which can be carefully established, and the results of the intradermal tests correspond quite closely with those of the subcutaneous test.

PATHOLOGICAL FINDINGS IN A CASE OF OCHRONOSIS

B. S. KLINE, M.D.

A case of ochronosis with melanuria was recently reported by Dr. B. S. Oppenheimer and Dr. N. W. Janney before the Section on Medicine of the New York Academy of Medicine. At present only a brief report of the pathological findings in this case will be given.

The patient, aged 40, male, presser by occupation, was admitted to the Montefiore Home and Hospital on November 18, 1915, having the clinical signs of ochronosis together with those of valvular disease of the heart. The patient died December 11, 1915, and was sectioned thirty-three hours after death.

PATHOLOGICAL REPORT

The autopsy revealed the lesions typical of ochronosis which will be but briefly described at this time.

The body is considerably emaciated. The skin in general is thin, sallow in appearance; in the arm pits it has a strikingly purplish-blue color. The cartilages of the ears are intensely blue in the inner portions, less intensely colored peripherally. The scleræ are tinted faintly blue except just below the cornea on each side where a much greater deposition of the pigment is present and in addition there is a wedge-shaped, greenish-blue area about 2×1 cm. The organs were removed through a rather small abdominal incision. The lungs, liver, spleen, pancreas, adrenals, bladder, seminal vesicles, and alimentary tract show no definite ochronotic pigmentation.

In the spleen there is a wedge-shaped, yellow opaque area reaching to the capsule about $2 \times 1\frac{1}{2} \times 1\frac{1}{2}$ cm. Near the apex of the infarct on section the tissue has a faint bluish tinge.

The heart weighs 360 grammes and shows dilatation of all the chambers with some hypertrophy of the right ventricle. At the base of one pulmonary cusp and at its attachment to the pul-

monary artery in two places, there is a bluish pigmentation of the endothelium. At one place above the auriculo-ventricular ring, the endocardium of the left auricle is covered by numerous small friable warty vegetations. The aortic leaflet of the mitral valve shows on its upper surface a number of small gray friable vegetations. The posterior cusp is strikingly altered, there is a large, irregular, friable mass along the line of closure and free edge, yellow in color and in part calcified. The vegetation continues down the associated chordæ tendineæ. There is considerable bluish black pigmentation of the intima and endocardium along the line of attachment of the cusps of the aortic valve. This pigmentation is also visible on the posterior aspect of the aortic leaflet of the mitral valve. There are a few patches of bluish discoloration in the intima of the coronary arteries just beyond their origin from the aorta.

The kidneys together weigh 500 grams, are definitely enlarged, and each measures $13\frac{1}{2} \times 8 \times 6$ cm. The capsule strips readily, revealing a smooth surface on which the stellate veins are prominent. In addition there are innumerable pin point, or somewhat larger, bluish black spots. On section the cortex is uniform in width, averages 8–9 mm., has a watery reddish appearance, streaked and dotted everywhere with brownish and bluish looking pigment. This pigmentation, however, is most marked in the medulla, especially about the papillæ. The striations in the cortex are not very distinct but are apparently fairly regular.

The prostatic urethra presents a striking appearance; there are stony bluish-black masses of pigment varying in size from pin point to grape seed, projecting into the lumen, in some places uncovered by mucosa. No areas of injection are present about these masses. The prostate is of average size and consistency. On section it contains a large amount of bluish-black pigment varying from pin point granules to several each as large as a pea.

The aorta shows numerous atheromatous streaks in the intima and at just about the commencement of the thoracic portion there is a region of more advanced sclerosis in which for an area of a sq. cm. there is considerable bluish-black pigmentation.

The tracheal and bronchial cartilages appear bluish through

the mucosa. On section, however, they appear brown. The costal cartilages present a striking appearance; they are hard and show an intense bluish-black pigmentation. The xyphoid also shows deep blue pigmentation. The ribs themselves show no apparent discoloration. The intervertebral discs show intense bluish pigmentation, they are more narrow than normal and in part invaded by bony tissue. The bodies of the lumbar vertebræ appear flattened. Macroscopically, the anterior ligment of the spine does not show any pigmentation.

Owing to permission for partial autopsy only, the larger joints could not be investigated. X-ray pictures of all the joints of the body, however, were taken. The spine shows changes characteristic of arthritis deformans. The larger joints (hip, knee) show well-marked changes, especially about the attachments of the capsules. The smaller joints show very little change (Scholz).

Histologically, the cartilages show the presence of the pigment in the form of fine granules in the matrix about the cells, and also in the perichondrium. The pigment is present in the form of granules in some of the cells of the tubular epithelium of the kidney, a few glomeruli likewise show a number of large mononuclear cells within the glomerular sac, all filled with fine brown pigment granules. In the sections of the kidney there is some distortion of the striations. In areas there is an increase in the interstitial connective tissue, in some of which accumulations of round cells in considerable number are seen. In some of these areas and elsewhere the glomeruli have an altered appearance, the tuft is bound to the capsule in one or more places by connective tissue. The ochronotic pigment is present in more diffuse form in the deeper layers of the endocardium and of the intima, in the renal tubules and in the corpora amylacea of the prostate.

The lesions observed in this case correspond closely to those in previously reported cases of ochronosis.

Discussion:

Dr. Ewing: It would seem possible that the early stages of ochronosis may sometimes be overlooked. I am very glad to have seen this case as I have never had the opportunity of examining the organs in this disease.

Dr. Kline: Virchow thought that up to a certain point the pigmentation was a physiological process and that the ochronosis was merely an overstepping of the physiological limits.

Dr. Ewing: Is there no general clinical guide as to the type of patient in which this condition occurs?

Dr. Kline: Some of the clinical signs are: Pigmentation of the scleræ, cartilages of the ears and skin of the armpits. Associated with these are signs of arthritis of the larger joints and spine, and frequently alcaptonuria.

Dr. Wood: Some cases may be overlooked clinically. Do cases of carbolic acid poisoning show alcapton in the urine?

Dr. Kline: In some cases the urine appeared normal. In some there were present phenol derivatives combined with ethyl sulphuric acid.

Dr. Zingher: What was the cause of death in this case?

Dr. Kline: Death was dependent upon the endocarditis.

ACTION OF RADIUM ON EMBRYO HEART MUSCLE

FREDERICK PRIME, M.D.

(From Columbia University, George Crocker Special Research Fund, F. C. Wood, Director)

During the course of a large series of investigations upon the action of radium on various tissues, it was found necessary, in order to complete the work, to observe this action upon embryo heart muscle. For this purpose the heart muscle from the chick embryo was selected as the most suitable, the points favoring its use being too well known to need more than a passing word. The ease with which it is obtained and kept living through an unlimited number of generations has been described at great length by Carrel and others; the fact that small pieces of this muscle will continue to beat for many days and weeks also had an important bearing on our work.

Our method was a simple one. The hearts were removed from chicks which had been incubated for seven days. They were then cut into small pieces, about 0.005 gm. in size, and suspended in a hanging drop of Ringer's solution, sealed over a deep slide. In each drop some twenty-five bits were placed. One of these preparations was kept for control observations, while the others were covered with 0.4 mm. brass and exposed to the

action of 100 mg. of radium for one-half and two hours respectively, these exposures having been found in other experiments which have been reported at length elsewhere, to be a sub-lethal and lethal dose when, as was the case here, both beta and gamma rays are employed. Each piece of tissue was then removed and planted in chicken plasma slightly diluted, to which was added an extract made from whole chick embryos. These were kept in an incubator at 37° C.; daily observations were made; and at the end of forty-eight to seventy-two hours the bits were removed from the plasma, washed in warm Ringer's solution, and replanted in fresh plasma and extract in which they would continue their life cycle. The results of the observations made and collected from a large series of these experiments, extending over many months, are quite consistent.

On studying the cultures at the end of twenty-four hours, there was found to be a profuse growth of all the plants made in vitro, whether treated or controls, and the muscle in all cases could be seen to beat most actively. Often it seemed that those tissues treated with a short exposure of radium showed a more profuse growth than the controls, but further observations will be needed to determine whether this is or is not a constant result. At forty-eight hours the growth was slightly better than at the previous observation, but the pulsations were not so active as they had been. The tissues were then washed and transplanted; and the second generation was studied at the end of twenty-four hours. The growth in these was found to be good in all the cultures and there was no marked difference between the treated specimens and the controls. The number of fragments which were pulsating, however, was generally less than in the original cultures. In forty-eight hours these were again transplanted and on observation twenty-four hours later there was seen in this third generation quite a marked difference. The controls still showed, as was to be expected, a good growth, and the same was observed in the cultures treated with a sub-lethal dose of radium; but in the cultures which had been exposed to the radium for two hours, there was a marked reduction in growth. As a rule, there would be found one or two specimens which produced a slight

growth; but in the vast majority of cases there was no growth at all, though many of those which showed no power of proliferation would still be beating, proving that they were at least alive. In the fourth generation no growths were found where a large dose had been administered, but still some fragments were pulsating; and in this generation there was a change in those cultures which had had a small dosage of radium. The specimens which showed growth were not so numerous nor were the growths which were present nearly so profuse as were those observed in the controls, and many plants could be seen to beat which showed no signs of growth.

In the fifth generation there was found to be practically no growth in any of the treated cultures, though many were beating; whereas the controls were growing and many more of them were found to be beating than of the radiumized specimens. From this point up to the ninth generation there was no change seen. The controls were growing and pulsating in many cases, while occasionally one was observed to beat without any growth; but growth was never found in the radiumized tissue, though several were observed to beat. When there was no longer any growth or pulsation, the tissues were discarded.

These results coincide very closely in most respects with the observations recently made and published by Dr. Wood and myself, in which it was found that a two-hour exposure to the beta and gamma rays killed tumor tissue so that it was unable to proliferate, whereas a half-hour did not always kill but simply retarded the power of growth. In the embryo heart, which is much more delicate than tumor tissue, it is seen that several days elapse before the action of the radium upon the proliferative power of the cells can be noted, and that finally the growth of the cells is inhibited, although the tissue itself it not killed. These observations correspond in many ways to those made by von Wassermann, in 1914, on the action of radium on tumor cells in mice. He concluded that the cells were not killed, but were rendered incapable of multiplication and that the apparent growth was an ameboid outwandering of cells whose power of mitotic division had been destroyed. Halberstaedter, in the same year,

found that by radiumizing trypanosomes in vitro without much filtration, he could eventually prevent them from reinfecting mice. Hertwig stated that the most important part of the destructive action of radium was on the nuclear mechanism of division and not upon the cell protoplasm, and showed the effect of radium upon the nuclear division in ascaris eggs. His views also are confirmed by our observations.

Discussion:

Dr. Wood: It is interesting to realize that large doses of radium can be given to a cell which is capable of functioning but does not normally divide, such as heart muscle; while the same dose to epidermis or cancer tissue in which rapid division is occurring will kill the tissue. This is one more evidence of the importance of the function of the nucleus in the question of radium action, as was long ago shown by Hertwig. Radium does not seem to destroy the cell body except in overwhelming doses. In ordinary cell life division takes place, when the dimensions of the cell have increased its bulk so that the relation of surface to cubic contents has diminished to such an extent that the necessary absorption of food stuff is interfered with. The cell then divides in order to increase its surface. Otherwise it would die, and this is what happens to tumor cells which have been given a lethal dose of radium.

Another interesting fact is that if you radiumize a cell in the resting stage, you stimulate it if a sufficient dose is given; whereas the same dose will kill the cell if the nucleus is undergoing mitotic division. This seems to be the explanation of the great variations in the destroying effect of radium, as observed in human and animal tissues. In some cases the tumor will show an extraordinary response to the lethal dose of radium; in other cases no such effect is observed. The most susceptible tumors are those in which rapid growth is occurring, if you exclude certain types such as basal cell epitheliomata in which the cellular balance between growth and regression is very delicate. After the death of the cells which occurs from the inability to divide, their absorption takes place and then we notice the radium effect.

Dr. Kline: Is there anything characteristic about the histological appearance of the cell destroyed by radium?

Dr. Wood: There is nothing especially characteristic in the morphology of cells killed by radium any more than by any other physical agent. The paralysis of the mechanism of nuclear division results in the formation of multinucleated or syncytial cells. These are very commonly met with in radiumized tissues, but are also found in tissues which have been *x*-rayed, and occasionally about areas of necrosis produced by other changes. Both radium and *x*-ray are apt to cause an extreme proliferation of the vascular endothelium in the smaller arteries, which sometimes enables a diagnosis to be made.

Dr. Bowles: I would like to ask Dr. Prime how he exposed the tissues to radium.

Dr. Prime: The heart of the young chick was suspended in a drop of Ringer's solution. It was then exposed to the radium, which was filtered through brass, which permits the beta and gamma rays to go through.

THE GROWTH OF HETEROLOGOUS TUMORS IN THE CHICK EMBRYO

HOLLAND N. STEVENSON, M.D.

(From Columbia University, George Crocker Special Research Fund, F. C. Wood, Director)

In the course of experiments upon certain biological phases of transplantable mouse and rat tumors, it was found advisable to extend the work to include the growth of these tumors in the chick embryo. This paper reports some of the interesting points demonstrated in the experiments. The technique for opening and inoculating the eggs was the same as that employed by Murphy, who first showed that heterologous tumors could be grown with success in the membranes of the chick embryo. It was not found necessary to use eggs from a single strain of fowls, as the tumors seemed to grow equally well in eggs from all strains. Incubation was maintained at about 103° F. The embryo was inoculated between the fifth and the eighth day of incubation, and the graft removed for transplantation between the sixteenth and the eighteenth day. The tumor was cut into bits of 0.01 gm. and inoculated through the opening in the shell with a pair of curved forceps.

Of the eggs inoculated, between 35 per cent. and 40 per cent. were found when opened to contain living embryos. Of those living, 50 per cent. to 75 per cent. bore tumors, the percentage usually varying quite widely with the series.

The tumors inoculated into the membrane of the chick embryo with resulting growth were the Crocker Fund rat carcinoma 10, mouse carcinoma 11, and mouse sarcoma 180, also the Ehrlich mouse sarcoma, and the Buffalo rat sarcoma. The Ehrlich sar-

coma was one of the tumors grown with success by Murphy. All these tumors grew well in the membranes of the chick embryo, and some have passed through several generations.

On opening the inoculated egg on the eighteenth day, the graft was found usually attached to the allantois near the site of inoculation; it was covered by the membrane which had grown around it and projected down into the allantoic cavity. In size, the graft varied from 2 to 6 mm. in diameter; it was quite firm and on section was translucent throughout, rarely showing areas of necrosis. On comparing it with the same tumor grown in mice or rats for an equal length of time, it was seen that the growths were about the same in size. The tumors in the animal, however, almost always showed some necrosis.

Microscopically, there was evidence of active growth. The tumor cells were seen wandering out into the mesenchyme tissue of the membrane, the sarcoma cells coming out singly, but rarely far from the main growth. The carcinoma cells extended out in groups, maintaining their general arrangement as in the original tumor. There was practically no change in the general appearance of these tumors during growth in the chick. The sarcoma cells possibly appeared larger; but this was very probably due to the difference in their immediate surroundings, for the mesenchyme tissue of the chick was much more delicate than adult connective tissue and pressure on the tumor cells must have been much less. Mitotic figures were present in abundance. A round cell reaction was always found around the growing tumor in the chick membrane, although this varied considerably in the number of cells present.

It was thought advisable to determine whether the characteristics of the Ehrlich sarcoma were modified in any way by a sojourn in the chick embryo. For this purpose a number of eggs were inoculated, and at the same time and from the same tumor, inoculations were made into a series of mice. The mouse series was used as a control and the growths were transplanted on the same day that transplants were made into mice from tumors growing in the chick embryo. It was found, on inoculating mice with a tumor grown in the chick embryo for one generation, that

the number of takes was smaller than among the control animals, but that the average weight of the tumors was greater than of those in the control series. On transplanting these tumors from mice into the next generation of mice, the number of takes was the same as in the controls and the average weight of the tumors almost the same. This showed that growth in the chick embryo had influenced this tumor in some way, but that this influence ceased on subsequent transplantation into mice. With a tumor grown for two generations in the chick embryo and then transplanted into mice, the number of takes was less than in the controls and also much less than in the mice inoculated with tumors grown for one generation in the egg. The average weight of the tumors of this series was much less than that of the tumors in the control series. This indicates more strikingly that growth of the Ehrlich sarcoma in the chick embryo influences its behavior when it is reinoculated into mice.

Discussion:

Dr. Weil: Is there any theory to account for this loss of virulence on retransplantation back into the original host? Murphy has retransplanted tumors but I do not think that he has made this observation. Has Dr. Wood any theories?

Dr. Wood: It is very interesting to speculate as to why the first generation of inoculations from egg to animal is larger than from animal to animal. The increased growth may be due to the fact that the piece which is transplanted from the egg is composed of actively dividing cells, as the lantern slides have shown, while the fragments of tumors direct from the animal often contain but few mitoses. It may be therefore that the piece from the egg gets a start, so to speak, which accounts for its larger size; but the second generation from animal to animal will go back to its normal growth rate as Dr. Stevenson has shown. The lowering of the rate of takes may be due to the fact that the rapidly dividing cells when removed from the chick embryo are more easily damaged by the trauma; and possibly the fact that the cells have fed upon chicken proteid instead of upon mouse proteid may render necessary a certain amount of readaptation to conditions of growth in mouse tissue.

LIMITATIONS TO THE CULTIVATION OF MAMMALIAN TISSUES IN VITRO

R. A. LAMBERT, M.D.

(*From the Pathological Laboratory of the Presbyterian Hospital, New York*)

A number of enthusiastic papers on the subject of tissue cultures, for some of which the writer was responsible, have undoubtedly give rise to erroneous impressions as to the possibilities of the method, especially regarding the variety of mammalian tissues which may be cultivated. It was, I believe, with the idea of correcting certain of these misconceptions that the President of the Society suggested the present report of my experience with the method.

I should like first of all to present a classification of tissues based on their behavior in vitro. Afterwards the factors which may possibly be responsible for failure in the propagation of certain of the tissues will be briefly considered.

Our experience has suggested that tissues may be grouped as follows:

1. Tissues showing no cell multiplication or migration in cultures. Microscopic sections indicate that the cells in the tissue fragments survive for a time. Examples are: highly specialized cells, such as liver and kidney epithelium, and ganglion cells; also those of human malignant tumors.

2. Tissues showing no multiplication but active wandering. The cells die and undergo disintegration after a few days' activity. Examples are: leukocytes, mononuclear wandering cells of spleen, lymph glands and bone marrow and epithelium of skin and cornea.

3. Tissues showing both active multiplication and migration which cease after a short time in spite of efforts directed toward continued propagation. Examples: malignant tumors of rats, mice, and fowl.

4. Tissues showing active multiplication and migra-

tion with tendency to progressive increase of growth in subcultures. Example: connective tissue cells of all species.

It is clearly important to know the normal behavior of various tissues in culture, as otherwise erroneous interpretations of experiments are apt to be made. Spleen, for example, was used by Carrel and Burrows,[1] in studying the influence of physical changes in the plasma medium on cell activity. The apparent stimulating effect observed has been referred to as an "activation of growth." Since the cells which migrate during the first two or three days in spleen cultures, are leukocytes and mononuclear wandering cells which show no evidence of multiplication, it is obvious that there could have been no true stimulation of growth.

Why certain tissues fail to grow and others exhibit only a brief period of growth in vitro is a question not readily answered.

Examining the list of tissues given above it is to be noted first that many specialized epithelial cells show only a brief survival. This is not surprising in view of the recognized susceptibility of such cells in the body to mechanical and toxic injuries, or even to mere disturbances in nutrition. Indeed it is rather remarkable that such sensitive elements as ganglion cells should survive for any considerable period. Also the temporary activity of leukocytes and related cells of the spleen, lymph glands and bone marrow, is not to be wondered at, since the life of these cells in the body is probably very short, and they do not reproduce themselves even in vivo.

On the other hand, it is surprising that the cells of malignant tumors of rats and mice should show only a brief period of activity in contrast to the prolonged, apparently unlimited, growth of connective tissue cells of those species; also that the cells of human malignant tumors cannot be cultivated by methods which allow the growth of normal cells and benign tumor cells.[2]

In seeking the explanation for these differences in the behavior of tissues in vitro several factors may be considered: mechanical injury, accumulation of toxic metabolic products, presence of inhibiting antibodies, and nutritional disturbances.

Mechanical injury. This factor is certainly important in

determining success in the cultivation of any tissue, as small experience with the method will show. But much evidence could be submitted, especially regarding the rat and mouse tumors, to prove that tumor cells are not particularly susceptible to traumatic injury. Besides, such injuries would explain only the failure of the cells to grow in primary cultures and would not account for the early cessation of growth observed.

Accumulation of metabolic products. In tissue cultures, put up according to the method in common use, there is no mechanism for the removal of waste products, or for the continuous supply of fresh nutritive medium. It is conceivable, therefore, that the accumulation of the products of metabolism may exert an inhibiting action on the growth of very active cells. In order to eliminate this factor as far as possible, early transfers have been made and the tissue fragments have been washed for varying periods in salt solution or hypotonic Ringer's solution as recommended by Carrel[3] and his co-workers who attributed their success in the prolonged cultivation of embryonic connective tissue largely to this procedure. We did not find that this treatment changed our results. It may be stated in this connection also, that, in the cultivation of adult connective tissue, an increasingly active growth was observed when the tissue fragments were transferred to fresh medium without washing.

It has been concluded, therefore, that the factor of inhibition by metabolic products does not serve to explain the failure of certain tissues to grow, or the limited growth of others.

Presence of inhibiting antibodies. While it has been established by various methods that antibodies for tumor cells do not exist in the circulating blood of rats and mice, it has been more difficult to settle this question for human tumors. Freund and Kaminer[4] have reported the discovery in human blood serum of two substances affecting tumor growth; one in the blood of patients suffering from cancer which exerts a protective influence on the cancer cells; the other in non-cancerous individuals which is lytic for cancer cells as demonstrated by test-tube experiments. While the experiments of these authors have not received con-

firmation by competent investigators, their work cannot be entirely disregarded.

In earlier experiments we used, as a matter of convenience, blood plasma from several normal individuals as a culture medium for human tumors. The negative results obtained and the work of Freund and Kaminer noted above, suggested that the plasma from the tumor-bearing individual might prove a better medium. It also occurred to the writer to see whether the presence of isoantibodies, agglutinins and hemolysins, influenced the growth in vitro of either tumor or normal tissue. This study has shown first that human carcinoma and sarcoma cells are not readily cultivated in vitro even in plasma taken from the individual carrying the tumor, while normal human connective tissue cells and benign tumor cells (mixed tumor of parotid) grow as well in homologous as in autogenous plasma. Furthermore the presence of isoantibodies is without demonstrable effect.

Nutritional disturbances. Before discussing this factor it may be well to consider the question advanced recently by Burrows as to whether cells growing in vitro can metabolize the food stuffs in the plasma medium. Burrows has expressed the view that the cells do not get nutriment from the medium at all. He assumes that they live on food carried over in the cell cytoplasm, a condition analogous to that seen in many developing ova.

In my opinion, a number of observations make this view untenable. In the first place, it is obviously impossible that a sufficient quantity of nutriment could be present in the cell cytoplasm to maintain active multiplication and wandering for any considerable period, whereas we know that active life in vitro, in the case of connective tissue cells at least, can be maintained for weeks, months or possibly years (Carrel). In the second place, if the cells did not take up nutriment from the medium succeeding generations should show a progressive diminution in size; but careful observation proves that this is not the case. Thirdly, the growth of cells in salt solution or greatly diluted plasma, where the food supply is markedly reduced, is of short duration and the cells of the second and third generation do become smaller. They show also very little fat accumulation as the result of the reduction in

the quantity of this food stuff in the medium.[5] It therefore must be admitted, I think, that cells do take up nourishment from the plasma in which they are growing.

If the failure of certain cells to grow is to be attributed to the food supply, the trouble must lie either in an inadequate supply or in the absence of certain specific food stuffs. That the quantity of nutriment is adequate is indicated by dilution experiments with those tissues which can be cultivated. Practically all such tissues will grow in plasma diluted with equal parts, or more, of salt solution without any effect on the rate or duration of growth.

Regarding the second possibility, namely, the absence of special food stuffs, it may be suggested that connective tissue cells can be cultivated for long periods in plasma of foreign species.[6] This result is opposed to the idea of strict food specificity. However, in the hope of supplying some such necessary hypothetical substances, extracts from various organs including liver, spleen, thyroid, testis, malignant tumors, and adrenal, have been added to the culture medium. No effect from such additions was observed.

CONCLUSIONS

Connective tissue is the only mammalian tissue which can be cultivated in vitro for any considerable period.

The transplantable tumors of lower animals show very active growth for a short time only. The previous reports of prolonged cultivation of such tissues were probably due to confusion between tumor cells and connective tissue cells in the culture preparations.

Human malignant tumors have not been cultivated successfully by us, although human connective tissue cells and wandering cells show marked activity in cultures.

The limited growth of animal tumor cells and the negative results with human malignant tumors may be referable to several factors, mechanical injury, accumulation of metabolic products, presence of inhibiting antibodies, and nutritional disturbances; but my experience leads me to believe that no one of these factors is alone responsible.

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Discussion:

Dr. Wood: A presentation of the difficulties in the growth of tissues in vitro such as Dr. Lambert has just given is most wholesome. Judging from the majority of the published reports, the growing of tissues, including cancer, is extremely easy; but in my laboratory we have found that human and animal tumors, on the contrary, grow with great difficulty despite the most careful technique. A full knowledge of the difficulties may lead easily to some improvements in this important biological method.

Dr. Ewing: At Buffalo, some years ago, some possible limitations of the growth theory of tissue cultures were mentioned. Although the presence of growth phenomena was not denied, it was suggested that the appearances might represent, in part, out-wandering of cells and prolonged survival.

Dr. Lambert: I understood Dr. Ewing to express the opinion at Buffalo that there was no growth of tissues in vitro. I have not taken that position at all. There is very active growth of certain tissues; some tissues show only migration of cells and others show only a temporary active growth. I emphasized the difficulty of cultivating human malignant tumors.

Dr. Ewing: I did not suggest at Buffalo that there was no growth in vitro but that the phenomena observed might be referable to a survival and migration of cells.

Dr. Wood: The real difficulty is to get continuous growth. It is easy enough to get some proliferation for a few days, the cells probably growing on their reserve supply of nutrition; but when these cultures are transferred to fresh plasma they usually refuse to grow further.

THE ANALYSIS OF SERUM SICKNESS

RICHARD WEIL, M.D.

It was suggested some years ago by von Pirquet that serum sickness is in some manner due to the interaction of antigen with newly formed antibody, in the blood. This theory was supported by the observation that precipitin may be demonstrated in the blood of the patient toward the end of the serum sickness. Two years ago I was able to show that these precipitins possessed the

function of passively sensitizing normal guinea pigs toward antigen,[1] thus demonstrating that anaphylactic antibodies play a rôle in the reactions of this disease. In addition, I was able to show that the antigen itself, namely horse serum, can be demonstrated in the blood even after the serum sickness has subsided. Within the past year I have studied a series of cases of serum sickness with the object of determining the presence of precipitin and of precipitable free antigen in the blood at various stages of the disease. Antigen was demonstrated by the reaction of human serum with the serum of a rabbit immunized against horse serum. The results of these studies have been summarized as follows: Preceding the serum sickness antigen is present in large amounts; the antibody is not demonstrable. Either at the beginning, or one or two days after the onset, of the serum sickness, antibody becomes demonstrable and increases in amount steadily for a period of one, or even two weeks after the serum sickness has subsided. Following this, the antibody is demonstrable in the blood for weeks or months. Antigen is present throughout the serum sickness and persists for a week or more after its subsidence, and then disappears. Thus antigen and antibody are both present in the blood of the patient during the manifestations of serum sickness and persist together for a week or more thereafter. Finally, the antibody alone remains, the antigen having entirely disappeared.

Parenthetically, it should be added that our present conceptions do not permit of the view that the factors which coexist in the serum correspond in the strict sense of the terms to precipitin and precipitinogen. In view of recent studies[2] such an interpretation is impossible. Horse serum is composed of a considerable number of antigenic substances, and as each one of these is thrown out of the serum by the accumulation of the corresponding precipitin only those partial antigens remain to which antibody has not yet been formed in excess. In accordance with this view, it is found that serum taken from the patient in the third week after the therapeutic injections gives a precipitate with serum of the same patient taken in the second week, although the former

itself contains partial antigens not yet neutralized by corresponding antibody.

The question arises, how do these observations bear upon serum sickness. It seems evident, in the first place, that the phenomenon cannot be due, as originally suggested by von Pirquet, to the interaction of antigen and antibody in the blood, inasmuch as both of these two factors are present for some time after the subsidence of the sickness. The second possibility is the interaction of the cellular antibody with the antigen. Such an interpretation would be in harmony with the theory that anaphylaxis is invariably a cellular phenomenon, and, moreover, with the observation that human cells possess antibodies which respond to the antigen exactly as do those of the guinea pig.[3] The cellular antibody might react either with cellular or with circulating antigen in the production of serum sickness. The time relationships permit of a probable conclusion upon this point. It is known from previous studies that neutralization of cellular antigen by cellular antibody begins about six days after the introduction of the antigen, and continues for a week or ten days. At the end of this period the cellular antigen has been destroyed. These facts, to which I drew attention in 1914,[4] have been thoroughly elucidated in a paper by Dr. B. S. Denzer which will shortly appear in the *Journal of Infectious Diseases*. It is evident, therefore, that the period of serum sickness closely corresponds with the period of neutralization of cellular antigen by cellular antibody in the guinea pig.

The question naturally arises, why circulating antigen does not unite with cellular antibody to further prolong the serum sickness. The answer to this question is found in the fact that the union of cellular antibody with free antigen, when it takes place gradually, and especially when the cellular antibody is already partly saturated, gives rise to no symptoms. On the other hand, the explosive combination of circulating antigen with cellular antibody produces a sharp anaphylactic response. In one of the cases studied in my series this reasoning was sustained by the following circumstance. The patient, who had received a series of therapeutic intraspinal injections for meningitis, and had recovered from

the ensuing serum sickness, developed a recurrence of the symptoms of the disease, requiring a fresh injection. Blood taken just preceding this injection revealed the presence both of antigen and of antibody. The fresh injection was at once followed by a recurrence of the serum sickness. Unquestionably, the sudden introduction of a large amount of antigen led to an explosive combination with the cellular antibody. The same process also explains the so-called "accelerated reaction" to antigen on the part of individuals who had, at some previous time in their history, received a dose of the same serum, and therefore had in their bodies cellular antibody which promptly responded to a fresh introduction of the antigen.

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ACUTE GLOMERULAR LESIONS FOLLOWING INJECTIONS OF BACTERIA INTO THE RENAL ARTERY

A. M. PAPPENHEIMER, M.D., H. T. HYMAN AND F. D. ZEMAN

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In the course of work planned to study the reaction of a previously sensitized kidney to general infections, it became desirable to investigate somewhat more closely the fate of bacteria introduced in large numbers into the kidney by direct injection into the renal artery. These preliminary experiments, upon which we wish now very briefly to report, seem to us to have a bearing upon the relation of bacteria to the lesions in acute glomerular nephritis.

The organism used in most of the experiments was a hemolytic streptococcus kindly supplied by Dr. Zinnser from his laboratory stock. It was of low virulence for rabbits, and as much as two agar slants could be injected intravenously without causing obvious symptoms. Fine emulsions in normal saline, freed from coarse clumps, were injected directly into a renal artery with a fine-gauge needle. A successful injection is followed immediately by a general blanching of the kidney, the color returning almost instantaneously after the needle is withdrawn. Hemorrhage from the puncture wound is in most cases very slight. The temporary disturbance of the blood supply is well borne, and in only a few instances have the lesions observed been of such a character as to suggest that infarction had occurred.

The kidneys were removed at varying times after the injection of living or killed organisms, and the histological lesions and the distribution of the bacteria studied in sections. Without giving at this time detailed protocols, we may describe briefly the general results of our observations upon the fate of the injected cocci, and the lesions produced. It was hoped that we should be able with this technique, to induce changes of a more chronic character, comparable to the subacute or chronic glomerular nephritis in man. We soon found, however, that the extreme frequency of spontaneous nephritis in rabbits (about 33 per cent. in our animals) made it extremely difficult to interpret the lesions of a more chronic character, and to bring these into definite relation with the injected bacteria. So far as our experiments go, however, we have as yet been unable to reproduce the later stages of acute glomerular nephritis in rabbits. Our report, therefore, deals only with the acute changes occurring within the first few days after the injection.

One of the most striking points noted was the rapidity with which the injected organisms were taken up by leucocytes, and possibly also by fixed cells. In a kidney removed five minutes after injection, for example, numerous cocci were found in the sections, and most abundantly in the glomerular capillaries, each tuft containing one or several clumps or chains. A close study shows that even after this short interval comparatively few of

these cocci lie free in the capillaries. They are found in or upon the cytoplasm of the polynuclear leucocytes circulating through the tuft. At times it appears as if the capillary epithelium also ingested some of the organisms, and this is undoubtedly true of the endothelial cells of the capillaries between the tubules. Cocci were found also in stellate connective tissue cells, and occasionally lying free in contact with the basement membrane of the tubules. None were found free in the capsular spaces of the tufts nor in the lumina of the tubules.

The opposite kidney, removed ten minutes after injection, showed the same picture. After fifteen minutes practically all the cocci appear to be intracellular, their distribution and number being about the same as before. At this stage histological changes, as one might expect, are insignificant, but one does find in some of the tufts an increased number of polynuclears.

After one hour following the injection of a living culture distinct histological changes are present. The cocci are still found in abundance, but they are entirely confined to the glomeruli. Many of the tufts, even with the low power, seem to be more cellular than normal; and this is found to be due to the presence within the capillary lumina of plugs of cells, principally polynuclear, often imbedded in a pink-staining finely granular matrix, which does not stain as fibrin, and appears to be composed of agglutinated platelets together with cell fragments. The organisms are practically all within these cells, and appear, many of them, to be undergoing intracellular digestion, as evidenced by their swollen and irregular shape and their imperfect staining. Aside from these cell fragment thrombi in the capillary lumina, only slight changes are observed. The capsular spaces are wide, and may contain a delicate granular coagulum, and occasionally a red blood cell or two. There is no severe hemorrhage and no exudation of leucocytes into the capsular spaces, nor is there any marked alteration in the epithelium covering the tuft or lining Bowman's capsule.

Two hours after injection of a similar dose very few cocci are present in any part of the kidney; those found are entirely intracellular and present only in the glomerular tufts. The

lesions are similar to those observed after one hour, with the addition that occasional loops are distended with hyalin thrombi.

In a kidney removed four hours and five minutes after injection of streptococci, distinct lesions were found in many of the tufts. The accumulation of polymorphonuclears is intense, giving the tufts an extremely cellular appearance, and quite evidently impairing the passage of the red blood cells through them. The leucocytes, as in the previous kidneys, are confined to the tufts, and are not present in the capsular spaces, which are free from exudate. There is no desquamation of the epithelial cells. A few glomeruli contain hyalin thrombi in one or more loops, and about these the nuclei are distorted and fragmented. The glomerular lesions are diffuse, practically every tuft being involved to some extent. In spite of this marked inflammatory reaction very few organisms could be demonstrated. Those found were in short chains or appeared within the leucocytes of the glomeruli; a few small clumps also were found in the larger veins.

The left kidney, injected with an equivalent dose of killed streptococci, showed lesions of the same character affecting at least 90 per cent. of all the glomeruli; in some of the tufts there was noticed in addition a swelling of the capsular epithelium and of the epithelium covering the tuft. The lesion differed also in the absence of thrombi, and careful search failed to show stainable bacteria.

Seven and a half hours after injection of living cocci, diffuse and intense lesions of the glomeruli are found. Many of the tufts are practically bloodless and collapsed, in which case the distended capsular space is usually filled with a granular coagulum. The capillaries are crowded with leucocytes, some of which occasionally find their way into the capsular space. In addition there are found thrombi in one or several loops. There is also a slight periglomerulitis, especially where the thrombosed loop comes in contact with the capsular wall.

A kidney examined twenty-four hours after the injection of living streptococci shows lesions more focal in character than those previously described. Both in the cortex and in the pyra-

mids, there are present definite suppurative foci with numerous organisms. About 60 per cent. of the glomeruli show a distinct lesion. These vary in intensity, affecting in some of the glomerular tufts only a single loop, in others the entire glomerulus and periglomerular tissue. In the most severe lesions the cocci form emboli completely filling the vessel, whereas in the less severely affected tufts the streptococci are present in small groups or chains, or even in single pairs. The slightest lesions noticed are hyalin thrombi distending a single loop of the tuft, with degeneration of the adjacent endothelial nuclei. The injury to the capillary wall at the site of the thrombus is evident from its swelling and hyalinization. The overlying epithelial cells may be desquamated, and where the thrombosed loop comes in contact with the capsular epithelial cells, the latter become swollen or cast off, and a definite fibrinous adhesion may be present. In other tufts there is a very dense accumulation of leucocytes both within the tufts, in the capsular spaces, and in the loose tissue about the capsule. There is no marked degeneration of the tubular epithelium, but in many places wandering cells are present between the tubules, and these may contain cocci.

The right kidney, which was injected at the same time with killed streptococci, also showed lesions of the glomeruli, but the reaction was less violent than in the kidney injected with living bacteria. There were no suppurative foci. Cocci are present in some of the glomeruli, in clumps of considerable size. There are, however, no definite bacterial emboli, and most of the organisms are definitely intracellular.

Since dead organisms could no longer be demonstrated after seven and a half hours, it seems probable that in this experiment living bacteria reached the right kidney through the general circulation. In another experiment the kidney, twenty-four hours after injection of dead cocci, showed relatively slight lesions.

The above observations seem to show that one can produce acute glomerular lesions of the intra-capillary type by direct injection both of living and killed streptococci into the renal artery. Ribbert, Baehr and Quimby, and Fitz have produced toxic nephritis by the injection of uranium nitrate, iodine, etc., into

the renal artery, but, so far as we are aware, no attempt has been made to study bacterial lesions by this method, which enables one to bring large numbers of organisms into contact with the renal tissue. The experiments throw some light on the fate of the organisms which are thus brought to the kidney. Even with the enormous numbers of cocci injected, it is found that after twenty-four hours practically all the organisms are disposed of, and even after eight hours no organisms can be demonstrated. The only exception appears to be in the kidney removed after twenty-four hours, in which persistence and multiplication of the organisms seems to have occurred. In several kidneys removed at intervals of from two days to several weeks no such persistence of the streptococci was observed.

We shall not describe the lesions in these kidneys in detail at this time, because the occurrence of spontaneous lesions has made it difficult to correlate them with the effects of the bacterial injections, but it seems certain that with the organism of low virulence used in our experiments the disposal of the bacteria takes place very rapidly. We have attempted to determine whether a large proportion of the organisms were excreted through the urine. In a rabbit in which the left kidney had been removed several weeks previously, the right renal artery was injected with a thick suspension of living streptococci, and cultures made by aspirating the urine of the bladder at five-minute intervals. The maximum number of colonies was obtained twenty minutes after the injection, but the total number of organisms recovered from the urine during the first hour was not over 400. Toward the end of the experiment only ten colonies per c.c. were obtained. In a second similar experiment the urine was practically sterile throughout, but the secretion of the kidney was evidently much impaired by the long continued narcosis.

Although these results cannot be taken quantitatively as indicating the rate of excretion of the bacteria, the experiments show, nevertheless, that under these conditions relatively few bacteria are passed out in the urine. This agrees with the histological findings, in that no cocci are present in the capsular spaces or in the tubules. That many of the injected organisms pass directly

through the kidney into the general circulation could be shown by taking cultures from the renal vein. Immediately following injection one c.c. of blood was found to contain innumerable colonies, and even after twenty minutes there were over 700 colonies per c.c. In a second experiment large numbers of streptococci were obtained in a culture from the renal vein made one hour and five minutes after the injection, so that the discharge of the organisms into the circulation appears to continue for a considerable period. It would seem, however, that the bacteria which are thus discharged into the renal vein do not continue to circulate in the blood but are probably retained in the lungs. Cultures from the ear vein, made about half an hour after the injection, show only a few colonies.

The bacteria which remain in the kidney appear to be largely deposited and arrested in the glomeruli. This is not due to mechanical obstruction, because they are frequently found here as isolated pairs or short chains which do not plug the capillaries. They are, however, rapidly taken up by circulating leucocytes, so that even after five minutes many of the bacteria are no longer free in the circulation. A few of the bacteria appear also to be taken up by the fixed cells of the tissues, being found in the endothelium of the capillaries, and also within the spindle shaped or stellate connective tissue cells between the tubules. This rapid phagocytosis would appear to explain the early disappearance of the organism from the lesions, and to make clear also why it is that it has always been so difficult to detect bacteria in acute glomerular nephritis, clinically of streptococcal origin.

Discussion:

Dr. Thalhimer: The work of Drs. Pappenheimer, Zeman and Hyman is very interesting. I should like to mention in connection with this paper some work, which is not yet published, done by Dr. Baehr and myself about a year and a half ago. We used the same technic for injection of the renal artery as used by Dr. Baehr in his previous work on experimental nephritis. This technic is the same as that outlined by Dr. Pappenheimer. *Streptococcus mitis,* or *viridans,* was used in our experiments. The object of the work was to study the lesions produced by the streptococci injected and especially to learn if embolic glomerular lesions similar to those found in cases

of subacute bacterial endocarditis are produced. In this latter we were successful. Streptococci were present in the portion of the glomerular tuft affected and remained there for a considerably longer time than did the hemolytic streptococci reported by Dr. Pappenheimer, *i. e.*, at least as long as nine days.

In a few other experiments which I performed *Staphylococcus aureus* or *Micrococcus tetragenus* were injected into either the portal vein or renal artery of dogs. These experiments were undertaken to find out if bacteria could pass through the normal kidney into the urine or through the normal liver into the bile. Occasionally a few bacteria were present in the bile but never in the urine. Incidentally it was found that the bacteria were present in great numbers in the general circulation for at least two hours after injection. They were therefore not filtered out by either liver or kidney.

I would like to ask Dr. Pappenheimer whether the hemolytic streptococci were found in his experiments in the glomerular tufts in the later stages of the lesions described by him.

Dr. Ewing: What was the final result in these cases? Was the picture of chronic nephritis ever produced?

Dr. Pappenheimer: In answer to Dr. Thalhimer's question, I am not able to say offhand whether organisms were present in these cases which showed thrombosis and necrosis of glomerular tufts.

Replying to Dr. Ewing I can only repeat what I said in my paper, that we are not ready to state what the final results of these lesions are. Any conclusion on this point has been especially difficult because of the common occurrence of spontaneous chronic nephritis in rabbits.

LESIONS PRODUCED IN SENSITIZED RABBITS BY INJECTIONS OF BACTERIAL EXTRACTS INTO THE RENAL ARTERY

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The purpose of the following experiments was to investigate the influence of previous sensitization to bacterial proteins upon the appearance of renal lesions, when bacterial extracts are brought into direct contact with the tissues of the kidney.

Rabbits were used for the experiments, and were given ten daily inoculations of increasing amounts of bacterial extracts. The extracts were prepared, by grinding the dried bacterial sedi-

ment obtained from mass cultures with sodium chloride crystals and glass sand, and then bringing to isotonicity with distilled water. In some cases sodium carbonate was added to an amount of 0.2 per cent. The determination of the actual sensitiveness of the animals was attempted by means of intradermal skin tests, but the results were most disappointing, as control animals reacted for the most part in the same manner as the treated ones. No other tests were attempted, and it was decided to regard the change in the kidney itself as evidence of sensitization.

About fourteen days after the last sensitizing injection, the animals were anesthetized, and given an injection of the bacterial extract directly into the renal artery. Forty-eight hours later, the animals were killed, and the kidneys prepared for examination. The frequency of spontaneous lesions, radial scars, and round cell accumulations in our rabbits, made it seem wiser to limit our observations to acute lesions.

The first experiments were with typhoid bacilli. The bacterial extracts used were not sterilized nor filtered, from fear of altering the bacterial protein, and were freed from bacillary masses only by brief centrifuging. This left small numbers of free bacilli and small masses of bacterial fragments in the supernatant fluid used for the injection. The kidneys of two treated rabbits, injected with 0.25 c.c. of such extract, show after forty-eight hours in the injected kidney—the opposite being entirely normal—glomerular lesions, corresponding closely in distribution and extent to those described by Löhlein and Baehr in subacute bacterial endocarditis. One in every six or seven glomeruli is affected, and shows changes in only one or in several loops of the tuft. The affected loops are bloodless and contain a very finely granular material, in which, especially about the periphery, there are polymorphonuclear cells and nuclear fragments. Occasionally a small loop contains a homogeneous thrombus with only one or two leucocytes, while in other loops the polymorphonuclears and nuclear fragments are more abundant, and in some tufts there is merely a diffuse accumulation of the leucocytes. The endothelial cells enclosing the smaller cell groups can often be made out distinctly, in other cases their

nuclei are pyknotic, and the cells appear to be a part of the degenerating cell mass. The *membrana propria* of some capillaries of this sort appears thickened and hyaline. The capsular epithelium does not show desquamation, and there is no exudate into the capsule. A very few capillaries outside of glomeruli, show similar changes to those within tufts.

Two control animals receiving injections of the identical extract died within forty-eight hours, and their kidneys were not examined. Similar amounts of two other typhoid extracts, made in exactly the same manner, were then injected into normal rabbits. In one the kidney appears entirely normal, in the other there are a few areas of polymorphonuclear accumulation in one portion of a glomerulus, and a single small loop was found presenting the same picture of nuclear fragmentation, etc., seen in the kidneys of the treated animals. There is a distinct difference in the extent and severity of the changes, from those in the treated animals.

The question at once arises, are these lesions merely embolic in origin, as claimed for those of subacute bacterial endocarditis? To determine this, rabbits were treated with typhoid, pneumococcus, and streptococcus extracts, and injected after an interval, as before, with bacterial extracts which had been centrifuged strongly, to free them from bacterial fragments that might act as emboli, and in which the bacteria had been killed. The kidneys of two typhoid, three streptococcus and five out of six pneumococcus rabbits so injected show no pathological change. It appears, then, that under the conditions of our experiment, the presence of bacterial bodies has been essential to produce the lesions.

It seems quite possible that the length of contact—not over thirty seconds—of the cleared bacterial extracts with the kidney tissue, was too brief to allow of any interaction between the two; and that the clumps of bacterial fragments in the extracts first used, while not acting as emboli, a conclusion suggested by the slight changes in the normal rabbits, at any rate allowed a longer contact and in more concentrated form, between the antigen and the tissues. To test this point and determine more sharply the

influence of sensitization, we then sensitized a rabbit with a single injection of egg white, and, fourteen days later, at which time the rabbit gave a positive skin reaction, injected the left kidney with 0.3 gm. egg white dissolved in 1.2 c.c. of 5 per cent. gelatin solution. It was hoped that the viscidity of this solution would delay its passage through the kidney and allow a longer contact with the tissues. At the same time the possibility of embolism seemed to be excluded, as the fluid was perfectly labile at 37° C.

The kidney of a normal animal injected with this egg-white solution appears entirely normal. In the injected kidney of the sensitized animal were found distinct glomerular lesions, which have again the focal distribution of the endocarditic renal lesions. One in about every ten glomeruli was affected, with involvement of one or several loops. The affected loops are plump and bloodless, and bear a close resemblance to those in the typhoid kidneys, although the polymorphonuclear accumulation and fragmentation is less marked, and there is greater evidence of endothelial swelling and proliferation. The capillary lumen is often filled with, or compressed by, swollen cells, apparently those of the endothelium. Where a capillary loop or an efferent vein is filled with a thrombus, apparently of platelet origin, the accumulation of polymorphonuclears and the nuclear fragmentation very closely resembles that in the typhoid kidneys. Capsular exudate and proliferation of epithelium were not noted, although about some capsules were small round-cell accumulations.

It appears from these experiments that a local anaphylactic or sensitization reaction may be produced within the kidney by intrarenal injection, and that this consists, at one stage, of glomerular lesions, with the production of cell thrombi and endothelial swelling and proliferation. Their focal distribution in the case of the egg-white reaction is difficult to explain. We have not studied the later stages. The results obtained with our typhoid extracts, which are primarily injurious even if to only a slight degree, require further study before they can be accepted as conclusive. Further experiments with guinea-pigs are in progress, and we hope here to test for sensitiveness by producing acute

anaphylactic death, and to employ the technic used in the egg-white experiments with cleared bacterial extracts.

THYROID AND PARATHYROID TUMORS OF THE TONGUE

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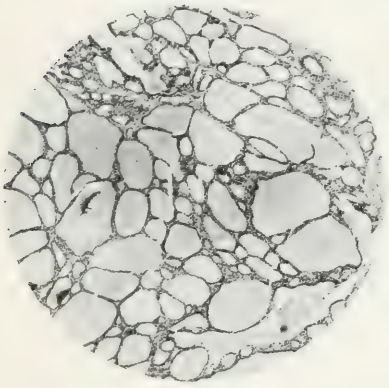
The presence of masses of thyroid tissue at or near the base of the tongue, while of considerable surgical and scientific interest, is a rather infrequent occurrence, only about one hundred cases having been published up to 1916. For this reason I wish to report the following cases which have been observed at St. Luke's Hospital. All were in the service of Dr. Frank S. Mathews, whose permission I have to use the clinical records.

Case I. H. O., female, age, 11 years. The child had always had a peculiarly husky voice from the time she learned to talk. About one year before admission she had a sore throat, and at that time her mother discovered an enlargement on the tongue, which had grown only slightly since. The child had never complained of pain or tickling in the throat, and except on the day of admission, when she vomited a little blood, there had been no record of bleeding. She was slightly defective mentally, but not myxedemic. Physical examination showed nothing of note, except that at the base of the tongue there was a rounded reddened tumor, seen best when the tongue was protruded. The growth was wholly attached to the tongue and apparently did not involve any other tissue. It measured about 2 cm. in diameter and when removed was found to be quite hard and filled with straw-colored fluid. There were similar collections of fluid in the wall.

When examined in the laboratory the specimen was found to consist of a cyst, with considerable connective tissue forming the wall. Microscopical examination showed the cyst to be com-

posed of loose connective tissue with numerous blood vessels and some hemorrhages. Evidently the cyst was not a true thyroglossal duct remnant, but was merely a simple cyst of thyroid tissue. In the wall there were a number of nests of thyroid gland structures (Fig. 1), a small nodule resembling parathyroid, and a good deal of muscle, probably derived from the tongue.

The finding of chief interest in this case was the fragment of tissue resembling parathyroid. A photomicrograph of this material is reproduced with photomicrographs of sections from two normal parathyroids taken without altering the position of the



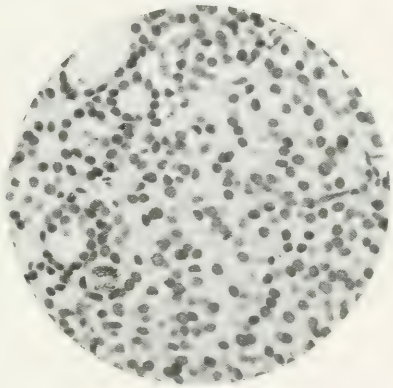


FIG. 1. Thyroid tissue from tongue. FIG. 2. Parathyroid tissue from tongue.

microscope and lenses (Figs. 2, 3, and 4), so that the magnification in all is exactly the same. The close resemblance of the three is evident.

Unfortunately the tumor was fixed in formalin, so that no satisfactory demonstration of glycogen could be made.

CASE II. A. S., female, age, 37 years. No history obtainable. At the level of the upper border of the thyroid cartilage there was a small, egg-shaped tumor, consisting of thyroid tissue, entirely separate from the main thyroid gland and not adherent to the surrounding tissue. At the base of the tongue, just posterior to the foramen cæcum, there was a small solid tumor about the size of a pea.

Microscopical examination of the piece of tissue from the

tongue showed irregular masses of thyroid scattered throughout the connective tissue. At one portion, the acini were much distended and filled with colloid material; in other places the glands were small. An area resembling parathyroid occurred in these sections also.

The neck tumor measured $5 \times 4.5 \times 3$ cm., and was smooth and round, and covered with a thin capsule beneath which were seen a few small blood vessels. Cut section showed firm, lobulated tissue, yellowish in color, which cut with a gritty resistance

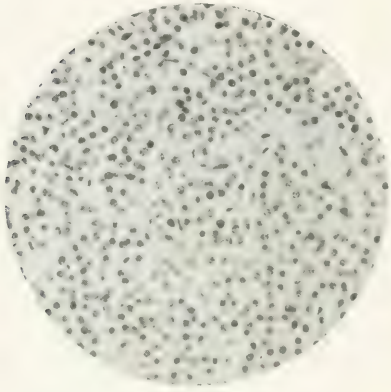


FIG. 3. Normal parathyroid.

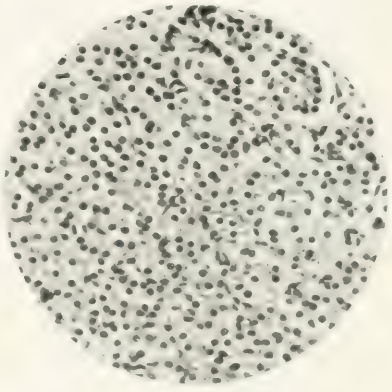


FIG. 4. Normal parathyroid.

and presented numerous small lobules and acini. Microscopically the specimen was composed of typical thyroid gland tissue.

These two cases are excellent illustrations of this rather rare condition. Neither have since developed myxedema.

A third case, which illustrates another phase of these tumors, is one in which the tumor was derived from the base of the tongue.

CASE III. H. W., female, age, 27 years. The patient had suffered from a lump in the neck ever since she could remember, which had remained about the same size and had given rise to no symptoms. On physical examination a small circumscribed and encapsulated mass was found on the right side of the neck at the level of the thyroid cartilage. This was freely movable. The thyroid was not palpable.

The specimen received at the laboratory was a cyst, measuring 2.5 × 1.5 × 1.5, roughly spherical, with a lobulated surface. Microscopical examination showed thyroid tissue with several large dilated acini surrounded by areas of thyroid tissue containing smaller acini. There are also remnants of a thyroglossal duct bearing ciliated epithelium (Fig. 5).

This type of tumor is much more frequent than that occurring on the upper surface of the tongue.

Fig. 5. Thyroid tissue about thyroglossal duct.

The following is a summary of the general facts known from examination of the cases heretofore published:

The usual site of the tumor is in the median line, occupying the area where the foramen cæcum is ordinarily situated. Sometimes, however, the thyroid tissue may be found at a short distance from this position on the dorsum or along the lateral border of the base of the tongue, but these cases are very rare. The tumor may be of small size, not over half a centimeter in diameter, but in some instances a mass the size of a lemon has been noted. It may scarcely project from the surface of the tongue

or may appear as a lobulated, in some cases pedunculated, mass at the base of the tongue, which greatly interferes with swallowing or speaking. The surface of the tumor is covered with mucous membrane and is usually smooth. The color is often a dark red or purple, due to the great vascularity of the structures beneath the membrane covering the growth. Severe hemorrhages have been noted following injury by the teeth or food. The differentiation between cysts, papillomata, and the malignant tumors which rarely occur in this region is easy. Some of the thyroid masses may be very firm, though not so hard as the chondromata that may occur at this site. In addition to the pathological interest connected with these misplaced thyroid gland masses, it is of surgical importance to remember that in about one-tenth of the cases the thyroid tissue in the tongue is the only example of that glandular structure in the body. Therefore, if the tongue tumor is completely extirpated the patient will develop myxedema.

In a case reported by Asch,[1] a patient after extirpation of a tumor at the base of the tongue developed not only myxedema, but also tetany; and on microscopic examination a number of small bodies resembling to a considerable degree the microscopic structure of the parathyroid were found. The presence of parathyroid bodies is difficult to explain from an embryological point of view, for while the median lobe of the thyroid is chiefly derived from the tissue which goes to form the tongue and springs from the point of junction of the second and third branchial arches, the base of the tongue is formed by two projections from these arches, which meet and form the foramen cæcum and the lingual duct. The latter extends down into the neck under the hyoid bone and inside the median thyroid lobe. The duct structure is lined in its upper portion by squamous epithelium, and in its lower portion by cylindrical ciliated epithelium. It usually disappears, but when it persists it is frequently surrounded by more or less scattered masses of thyroid tissue. The parathyroids are of a slightly different origin; ordinarily they are seen to arise from the pouches of the third and fourth branchial clefts, which are in close relationship to the

tissue from which the lateral lobe of the thyroid originates. The parathyroid bodies are usually found in or on the surface of the lateral portions of the thyroid gland itself, and parathyroids are not seen in connection with the central portion of the gland. A recent investigation of new material has suggested that possibly the second branchial pouch may give rise to some parathyroid tissue which would explain the presence of parathyroid in and about the tongue region.[2]

The only other interesting clinical feature attached to these tumors is that they occur almost entirely in women, only 12 per cent. being noted in men. Microscopical examination of the tissue of such masses shows either perfectly normal thyroid tissue with follicles filled with colloidal material or a rather rudimentary gland with follicles not containing any colloid, or a regular cyst-like cavity such as is seen in goitrous conditions of the thyroid gland itself. Hemorrhage may occur and softening, as is frequently seen in enlarged thyroids in the usual anatomical situation.

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Discussion:

Dr. Coulter: I would like to inquire as to the embryological explanation of the presence of parathyroid tissue in this region so far removed from the normal situation of the parathyroid.

Dr. Ewing: I have just looked over Dr. Wood's section and think it is impossible to say whether one has to do with young thyroid tissue or parathyroid tissue.

Dr. Wood: While it is generally stated by embryologists that the parathyroids come from the third and fourth pouches, a recent author has claimed that they may develop also from the first pouch, citing in support of his view a case similar to the one reported to-night. I agree with Dr. Ewing that it is difficult to say whether we are dealing in this case with both parathyroid and thyroid tissue or with thyroid tissue alone. It may be pointed out, however, that it is hard to understand why some of the thyroid tissue resembles the adult type, while other tissue in the same specimen is distinctly embryonic. It seems likely that we have to do with both parathyroid and thyroid, as the resemblance of some of the tissue to normal parathyroid is very close.

THE BIOLOGICAL EFFECTS OF RADIUM ON TUMOR CELLS

FREDERICK PRIME, M.D.

(From the George Crocker Special Research Fund, F. C. Wood, M.D., Director)

There has been much discussion of late as to the penetrating power of radium rays, and the efficacy of these rays in the treatment of malignant growths situated at some distance from the surface.

Two series of experiments were undertaken to determine the effect of radium on animal tumors. In the first, the tumor to be radiumized was removed and exposed in a moist chamber to the radium. At the end of the exposure, these pieces of tumor, together with the control which had been kept under similar conditions, were inoculated into mice or rats, as the case might be. The tumors were charted at weekly intervals, and from the growths which appeared, successive generations were propagated. When the gamma rays were used either in small or in large doses, it was found that, after the first generation, the tumors which had been exposed to small doses of radium for a short time produced larger daughter growths than did the controls, larger also than those which had been exposed to a dose of radium just under the lethal point.

A second series of experiments was conducted upon tumors in vivo, a mouse carcinoma being used for this experiment. Here the mouse was anesthetized and the skin over the tumor reflected. Then after a small portion had been removed from the tumor and retained at the same temperature as a control, the radium, filtered through 0.4 mm. of brass was applied to the tumor. This filter passes the gamma and all except the softer groups of beta rays. Exposures to 83 mg. of radium element for two hours were made. After the application of the radium, some of the tumor was removed from the area immediately be-

neath where the radium had been, and at the same time tissue from the other side of the tumor, which was directly beneath the radium tube and never more than 1 to 1.5 cm. distant, was removed. Both of these pieces of tissue, together with the control tissue, were inoculated into a series of mice, and in some cases subsequent generations were carried on. After the first generation, the tumors arising from the tissue 1 cm. distant from the radium, showed an increase in size as compared with the control and with those grown from the tissue immediately under the radium. This stimulation effect often lasted through eight or nine generations of transplants.

From these experiments it is concluded: First, that the action of the gamma rays from radium in small doses upon tumor cells in vitro not only does not kill, but has a stimulating effect upon the cells, and that this effect persists through several series of generations of transplanted tumor.

Second, that the action of considerable quantities of radium upon tumor tissue in vivo, is effective only after long exposure, and then only upon the tissue directly beneath the radium. There is often a stimulating effect upon tissue 1 cm. distant. This points to the necessity for employing very large quantities of radium, 1 gm. or more of the element, if any lethal effect is to be obtained on cells lying more than 1 cm. from the active agent.

Discussion:

Dr. Pappenheimer: How does Dr. Prime explain the diminution in weight of the controls in the second and third generations? His charts seem to show quite marked variations in the rate of growth of the controls, as well as in the treated animals.

Dr. Ewing: I agree with Dr. Pappenheimer that in these experiments the controls are unsatisfactory. In one set the controls grew more than the treated specimens but in most of the others the controls hardly grew at all, so that any comparison with them seems unjustified. Would it not be possible to secure a tumor which will grow more uniformly?

It has frequently been stated that radium treatment may be followed by more rapid growth of the tumor. I have been unable to reach a conclusion whether in such cases the tumor cells have been stimulated, or the inflammatory reaction causes the tissues to swell, or the tumor merely goes on its natural course. The experimental stimulation of the mitotic process by

radium has been proven only for very minute doses of radium. Clinically one observes that extensive carcinomas may continue to grow rapidly after inadequate treatment by radium, but it is difficult to say whether the growth has been stimulated. Recently I have seen a very extensive epithelioma of the lip which grew rapidly after radium treatment and it was feared that the growth had been stimulated. Later another patient with a similar tumor was admitted to the hospital, and on account of the previous experience it was not treated, but the same very rapid growth was observed. It is therefore of much importance that experiments such as those of Dr. Prime should be undertaken to test the matter, but even under experimental conditions it is not easy to reach a conclusion.

Dr. Levin: I should like to cite an instance similar to Dr. Ewing's of a patient referred for treatment, suffering from a spindle cell sarcoma of the upper jaw. Radium therapy was advised but the patient did not return until four weeks later, when the tumor had increased six times in size. In this case obviously radium was not responsible for the increase in growth.

Dr. Wood: The discussion of this question shows clearly that the way to get an answer is not by studying individual cases but by carrying out experiments on such a large scale that accidental results can be eliminated. Regarding the variations in the rate of growth of control animals, it is known that tumors grow better in young than in old mice, and better in animals coming from one section of the country than in those from another section. In our experiments we have been careful to take these differences into account, but in spite of this care, differences in tumor growth do occur, as has been noted. Such variations make necessary the use of large numbers of animals. When many experiments are completed, it may even prove necessary to call in a statistician to help interpret the results. It is, however, obviously a matter of great importance to determine whether tumor growth can or can not be stimulated by the use of radium, and, to my mind, the question can be decided only by studying large series of animals, not by a consideration of individual cases.

Dr. Prime: There is no explanation for the variations in the rate of growth of tumors inoculated into a series of animals; but the point I wish to make is that if a hundred animals, all of about the same age and from the same breeder, are inoculated at the same time with the same amount of tumor tissue, the average weights of the tumors in groups of twenty-five or fifty animals will be very close. When inoculation is made from one of the tumors of this series into a second series, the average weights of the tumors may vary in different generations, but all the tumors in the same generation will be of about the same weight. The only way to obliterate these biological differences is to use very large series of animals, and thus show that such accidental errors can be eliminated while the variations following exposure to radium are constant.

THE RELATION BETWEEN VITAMINES AND TUMOR GROWTH

ISAAC LEVIN, M.D.

(From Columbia University, George Crocker Special Research Fund, F. C. Wood, M.D., Director)

It seems plausible to maintain, a priori, that there exists a certain relationship between the growth of a malignant tumor and conditions of nutrition within the organism of the host, and indeed there exists a certain amount of research which seems to corroborate it.

Ehrlich's[1] hypothesis of athrepsia gave the first impetus to this line of investigation. In accordance with this conception a tumor graft when inoculated into a new host must obtain there a certain specific food-stuff. When the supply of this so called *x*-stuff in the organism is exhausted, either due to a deficiency of the diet necessary to furnish this specific *x*-stuff of food, or through a greater avidity of the normal body cells to the specific food-stuffs, then the graft fails to develop. The indirect experimental proofs of this hypothesis have no bearing on the present investigation and will not be discussed here, but a certain number of direct experimental studies will be analyzed.

Moreschi[2] in an attempt to furnish a direct experimental proof of the hypothesis of athrepsia studied the relationship between conditions of nutrition and the growth of an inoculable mouse sarcoma. He found that general underfeeding of the animal retards, to a certain extent, the development of a tumor graft. Similar results were reported by Haaland,[3] Stahr,[4] and Medigraceanu,[5] though their methods of research were not always as direct. Stahr for instance found that the difference in the takes of tumor grafts in different races of mice is due to variations in the feeding.

Rous[6] on the other hand obtained results which were not in complete accord with the previous investigations. He found

that a quantity of food sufficient to sustain the life of the animals, but permitting of gradual emaciation, may influence the development of a tumor graft but does not impede the subsequent growth of a large tumor. Furthermore he found that different tumors react differently to the underfeeding of the animal. Thus, Flexner-Jobling carcinoma of the white rat does not seem to be influenced at all by low diet.

All the subsequent investigations were conducted, not on a generally restricted diet, but, on feeding with specialized foodstuffs. J. E. Sweet, E. P. C. White, and G. J. Saxon[7] took as the basis for their research the investigation of Mendel and Osborne.[8] The latter have devised a diet which while sustaining the life of young animals inhibits their growth. Sweet, White, and Saxon argued that, since body growth and tumor growth are analogous phenomena, Mendel-Osborne diet should impede the growth of a tumor graft. They experimented with a modification of Mendel-Osborne diet, and seemed to have obtained positive results, *i. e.*, the successful takes of tumor grafts was markedly reduced in animals fed with this special diet.

Van Alstyne and Beebe[9] also claim that qualitative differences in food influence the growth of malignant tumors. Their experiments consisted in addition of sugars to an otherwise normal full diet, and they found that this addition of sugar enhances the tumor growth while lack of sugar impedes it. Woglom[10] was unable to confirm these results. In his recent study on the subject Rous[11] attempted to approach more closely conditions as they obtain in human cancer. His aim was to observe whether a special diet would retard the appearance of a local recurrence and the formation of metastases after an incomplete surgical removal of the primary tumor. He employed for the experiments a Bashford mouse carcinoma, No. 63, Tyzzer's carcinoma of the walzing mouse, Flexner-Jobling carcinoma of the rat and spontaneous tumors. Sweet's modification of Mendel-Osborne diet was given to all the animals. The diet apparently checked the development of the recurrence in some of the tumors, but did not affect the Flexner-Jobling tumor.

The results of all the investigations enumerated above do not appear to be sufficiently uniform or constant. On the other hand recent investigations on the etiology of beriberi and similar so-called deficiency diseases made very probable the existence in the food of a class of substances which C. Funk[12] named vitamines and which are of vital importance to the animal organism.

Vitamines are complex nitrogen containing crystalline substances, the exact chemical structure of which is not yet known. Deficiency diseases appear to be due to consumption of food which lacks the vitamines. Highly polished rice presents a food completely void of vitamines, while the rice-polishings contain all the vitamines of the rice. People living in the tropics and subsisting on polished rice as the only article of food suffer from beriberi and other deficiency diseases. An introduction into the organism of a small quantity of vitamine may cure a deficiency disease. An alcoholic extract of yeast is the most easily obtainable and the most active vitamine. Pigeons and chickens fed on polished rice emaciate, ultimately refuse to eat the rice and die of starvation. They also frequently develop a polyneuritis, which is an experimentally induced disease analogous to beriberi.

In view of the great importance of vitamine as a life-sustaining ingredient of food which moreover acts in minute quantities, it seemed plausible a priori, that of all the foodstuffs it should exercise the greatest influence on tumor growth, if the latter depends on conditions of nutrition at all. The writer, therefore, undertook to investigate the relation between vitamines and tumor growth. The sarcoma of the fowl described by Rous[13] is singularly well adapted for this line of research. The tumor is very malignant clinically, frequently forms metastases, and is readily inoculable in chickens. The latter animals on the other hand, as stated above, are easily influenced by changes in the vitamine contents of the food.

The experiments were conducted in the following way: 200 chickens divided in three groups were used for the experiments. The first group of animals were inoculated with the tumor simultaneously with the beginning of rice feeding. The second

group of animals were fed with rice, and at the same time 20 c.c. of an emulsion of yeast extract in water (5 gm. of yeast extract in 20 c.c. of water) were introduced into the stomach of each chicken through a catheter every other day. The yeast extract was kindly prepared by P. A. Levene, of the Rockefeller Institute for Medical Research. Four weeks after the beginning of the rice and yeast feeding the animals were inoculated with the tumor. The object of this group of experiments was to enable the tumor inoculation to take place in animals already suffering from a deficiency of vitamine. The additional yeast feeding as conducted in this investigation does not cure the rice-fed animals completely, but prolongs their life. Instead of four weeks they could be kept alive nearly seven weeks. Nevertheless the animals became severely emaciated, and even a greater percentage of them developed polyneuritis than of the animals fed with rice only. This is probably due to the fact that the latter did not live long enough to develop the disease. The third group of animals was used as control to the other two. They were given normal food and were inoculated with the tumor usually on the same day and from the same source as the corresponding series of animals of the first or second group.

Chart I shows the result of an experiment on a series of fifteen animals of the second group and of fifteen controls. The first, second, and third vertical columns of the chart are self explanatory. The animals were killed twenty-one days after inoculation, since by that time the emaciation was extreme and they entirely refused the rice feeding. On the other hand the fowl sarcoma generally reaches its largest size at the end of three weeks. The cross, figure, and letter "D" to the left of the fourth column indicates the number of days of life after the inoculation, of animals which died before the end of the three weeks. The word "poly" to the right of the fourth column indicates an animal which developed polyneuritis. The letters in the fifth column indicate the size of the developed tumors. The tumors were always inoculated into the breast muscle of the fowls. Letter "E" indicates that the tumor infiltrated the *entire* breast muscle. Let-

ter "L" means a *large* tumor the cut surface of which is not less than 10 cm. square. Letter "M" means a *medium* sized tumor the cut surface of which is not less than 5 cm. square. Letter "S" indicates a *small* tumor the cut surface of which is less than 5 cm. square. The dashes in the sixth column indicate the absence, and the crosses the presence, of metastases. The

EXPERIMENT IV B

RICE PLUS YEAST FEEDING						CONTROLS		
NO	INITIAL WEIGHT	WEIGHT ON INOCULATION 24 DAYS LATER	WEIGHT AT DEATH 21 DAYS LATER	SIZE OF TUMORS	METASTASES	NO	SIZE OF TUMORS	METASTASES
1	1900 GM	1600	†19 D 1300 POLY	E	+	1	S	—
2	2500	2225	†11 D 2075	S	—	2	M	+
3	2375	1950	1700	E	—	3	E	—
4	2050	1950	1800	E	—	4	E	—
5	1950	1975	1700	M	—	5	E	—
6	1800	1150	†14 D 975	M	—	6	E	+
7	1650	1375	†14 D 900 POLY	S	+	7	E	+ +
8	2175	1925	1575	E	+++	8	E	—
9	1925	1250	†11 D 825	S	—	9	S	—
10	1975	2200	1975	E	+	10	E	+
11	2150	1750	1325	E	++	11	E	+ +
12	1950	1650	1425	L	+	12	E	+
13	2075	1575	†12 D 1100 POLY	M	—	13	S	+++
14	2200	1625	1325 POLY	E	+	14	S	—
15	2150	1325	†14 D 950 POLY	S	—	15	E	+ +

CHART I

number of the crosses on one line shows the number of organs in which metastases were found in the animal.

An analysis of the chart I shows that there is no distinct difference between the behavior of the tumor in the animals fed with rice plus yeast and in the controls. The number of animals with large tumors and metastases is somewhat greater in the control series than in the fed animals, but this difference is within the limit of normal differences within various series of animals

taken at random. The same indefinite results were obtained in all the other series of experiments, indeed in some series the tumor appeared to grow better in the fed animals than in the controls. Extreme emaciation of the animal accompanied by the development of polyneuritis did not seem to prevent the growth of a large tumor and formation of metastases.

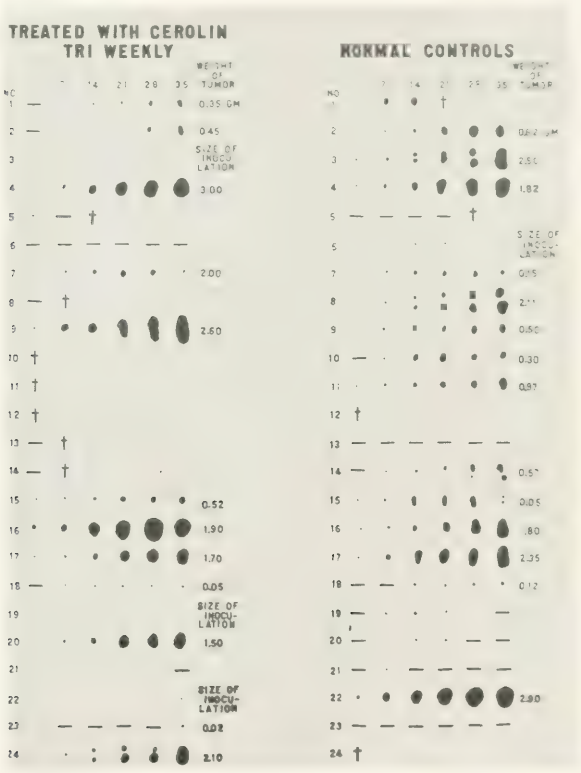

Chart II

Another mode of procedure to gauge the influence of vitamines on tumor growth employed in this investigation consisted in the introduction into the animal of additional quantities of the substance during the development of the tumor.

For these experiments a mouse carcinoma, No. 15 of the

Crocker Laboratory, was selected, because it grows comparatively slowly and gives approximately the same number of takes in the different series of animals. These characteristics of the tumor allow a sufficient time for the vitamines to influence the growth, and permit of a comparison between the number of takes and the size of the tumors of the treated animals and the controls.

Chart II presents a graphic description of one series of experiments: 48 mice were inoculated subcutaneously with 0.01 gm. of mouse carcinoma No. 15; 24 animals were injected three times a week subcutaneously with 3 mm. of a 10 per cent emulsion of cerolin in water. The latter is a commercial alcoholic extract of yeast. The other 24 animals were not treated and served as control. An analysis of the chart shows that the injection of cerolin did not enhance in any way the growth of the tumor. In fact the control series showed a greater number of animals with large tumors than the treated series.

Thus the growth of Rous's sarcoma of the fowl and of the carcinoma No. 15 of the mouse is not influenced by the variations in the amounts of vitamines taken in by the organism. While it is still feasible to presume a priori that variations in the conditions of nutrition of the animal may influence the growth of a tumor, even the finest experimental methods at our command at present cannot demonstrate such an influence. The metabolic differences between a normal body cell and a cancer cell are too finely adjusted to be detected by such comparatively crude methods as a quantitatively or qualitatively inefficient diet or even changes in the vitamine supply of the organism.

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SKELETAL METASTASES OF CARCINOMA

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A correct estimate of the frequency of skeletal metastases in malignant tumors is very difficult since the condition may exist for a long time without presenting any clinical symptoms or anatomical deformities. Moreover a routine autopsy in cases of malignant tumors as a rule does not include an examination of the bones unless there is present a distinct change in the configuration of a bone or a pathological fracture. Fraenkel[1] made a statement in a short society report on the subject of skeletal metastases, that the latter are found in about 20 per cent. of cases of carcinoma. Fischer-Defoy[2] in an analysis of 82 cases of carcinoma found skeletal metastases in 25.7 per cent. of the cases. Schmorl[3] reported that in a series of cases of carcinoma in his department, many bones were dissected and the marrow opened, even where there was no gross indication of any lesion. This investigation showed the presence of metastases in about 6 per cent. greater frequency than was found by Fraenkel and Fischer-Defoy.

An *x*-ray examination of the skeleton of cancer cases before or after death would easily reveal the presence of discrete metastatic nodules and obviate the necessity of sawing open all the bones of the body. A minute island of cancer cells in the center of the bone-marrow cannot be demonstrated on an *x*-ray plate,

neither is it of any value for a statistical estimate of the frequency of skeletal metastases. On the other hand these minute nodules in the bone-marrow are usually found in the neighborhood of large metastases, and therefore for the purpose of a microscopical study of the early stages of metastasis formation, it is unnecessary to open all the bones at the autopsy and search for small metastases.

During the last three years the writer has had the opportunity to study 11 cases of skeletal metastases. The primary tumor was located in the breast in 7 cases, in the thyroid in 2 cases, and in the lung in 2 cases.

The investigation consisted in the first place of a Roentgenographic examination of the parts of the skeleton, in which the clinical symptoms indicated the possibility of the presence of a metastasis. In certain cases repeated *x*-ray examinations were made in order to study the changes in the development of the metastatic tumor. Furthermore, a microscopical study was undertaken of the skeletal metastasis of the cases in which an autopsy was obtained.

The following are brief histories of the cases.

Case I. Mrs. A. S., age 54. Admitted to the hospital Nov. 20, 1913. Primary carcinoma of thyroid, metastases in the lungs and ribs. No satisfactory *x*-ray pictures were obtained. Died March 18, 1914. The metastases in the ribs served for pathological study.

Case II. Mrs. M. G., age 43. Admitted to the hospital Dec. 3, 1913. An amputation of the breast had been done two years previous to the admission to the hospital. Patient died on Dec. 31, 1913. Autopsy showed metastases of carcinoma in the viscera and in the sternum. The metastasis in the sternum served for pathological study.

Case III. Mrs. B. H., age 41. Admitted to the hospital Jan. 21, 1914, with an inoperable carcinoma of the right breast, painful spine and inability to walk. Clinical and *x*-ray study showed metastases in the eighth, ninth and tenth ribs, and in both femurs. Patient died May 20, 1914. Autopsy showed carci-

noma of the breast and metastases in the axillary lymph glands, pleura, both trochanters and the eighth, ninth and tenth ribs. A microscopic study was made of the ribs and trochanters.

Case IV. Mrs. T. S., age 39. Admitted to the hospital May 14, 1914, with a recurrence of a carcinoma of the right breast, which was amputated about six months previously. Clin-

1 2

Fig. 1. *X*-ray plate of the right femur of Case V, showing metastases.

Fig. 2. Photograph of the right femur of Case V, showing metastases in the bone-marrow.

ical and *x*-ray study revealed metastases in the right femur and clavicle. Patient died June 17, 1915. Autopsy showed medullary carcinoma of the breast, metastases in the axillary, cervical, mediastinal, bronchial and retroperitoneal lymph glands and in the head of the right femur. The metastasis in the femur served for pathological study.

Case V. Mrs. F. E., age 56. Admitted to the hospital Nov. 10, 1914, with a recurrent carcinoma of the breast which was amputated in 1913. Three weeks after the operation, patient felt pain in the right thigh. Clinical and *x*-ray study revealed metastases in both thighs. One month after admission the patient sustained a fracture of the right femur at the level of the metastasis. The patient died on Jan. 1, 1916. Autopsy showed metastases in the liver, ribs and both femurs. Figs. 1 and 2 show an *x*-ray plate and a photograph of a sawed-open surface of the bone-marrow respectively. It is very striking that both bones showed the metastases practically in the same positions. The metastases in the femur and ribs served for pathological study.

Case VI. Mrs. A. B., age 45. Admitted to the hospital Oct. 1, 1915, with a recurrent carcinoma of the left breast. The breast was amputated about six months previously. The patient complained of pains in the extremities which began half a year before the operation. An exhaustive *x*-ray study of the skeleton of the patient was undertaken and it showed the presence of metastases in practically every bone. The patient died on Jan. 22, 1916. No autopsy could be obtained.

Case VII. Mrs. D. L., age 40. Admitted to the hospital Nov. 10, 1915, with a recurrent carcinoma of the right breast, which had been amputated six months previously. Two weeks after the operation the patient began to feel severe pain in the spine and became unable to walk. *X*-ray examination revealed metastases in the second and third lumbar vertebræ. At the time of writing the patient is still in the hospital. Subjectively the patient is better, she is able to walk about and a subsequent *x*-ray examination shows new bone formation in the region of the metastasis between the second and third lumbar vertebræ.

Case VIII. Mrs. B. S., age 56. Admitted to the hospital July 6, 1915, with primary carcinoma of the lung. The patient died July 25, 1915. Autopsy showed adenocarcinoma of the lungs, metastases in pleura, liver, adrenal, ribs, vertebræ and in the lymph gland of the left axilla. The metastases in the ribs and vertebræ served for the pathological study.

Case IX. Mrs. R. R., age 43. Admitted to the hospital Jan. 16, 1916, with recurrent carcinoma of the breast which had been amputated about six months previously. About two months after the operation the patient began to suffer pain in the legs and back. Clinical and *x*-ray examination showed metastases in the eleventh and twelfth dorsal vertebræ, and the neck of the right femur. Patient died Feb. 13, 1916. Autopsy showed carcinoma of the breast with recurrence in wound and metastases in regional lymph glands, liver, pancreas, lungs, vertebræ and right femur. The metastases in the bones were studied microscopically.

Case X. Mrs. B. K., age 52. Admitted to the hospital Dec. 16, 1915. Died May 6, 1916. Autopsy showed carcinoma of the thyroid, metastases in the lungs and in the fourth lumbar vertebra. The latter was studied microscopically.

Case XI. Mr. K. R., age 62. Admitted to the hospital April 11, 1916, complaining of pain in the chest and coughing. His disease began seven months previous to admission. Clinical and *x*-ray examination showed a carcinoma of the lung. On July 29, 1916, there developed a tumor in the right clavicle. An *x*-ray examination showed destruction of the bone tissue and a pathological fracture.

The predilection of certain carcinomata to metastasize in the bones was ascribed to mechanical causes by von Recklinghausen,[4] who was the first to thoroughly investigate the subject. He maintained that the formation of metastases in the bones is due to the fact that the veins and capillaries of the bone-marrow have thin walls, and are not collapsible. These morphological peculiarities favor the accumulation of carcinoma cells within these blood vessels. The majority of pathologists side with von Recklinghausen in the opinion that the mechanical peculiarities are the main, if not the only factors in the causation of skeletal metastases. None the less this hypothesis is inadequate to explain all the phenomena connected with the formation of skeletal metastases. E. Kaufmann[5] gives a detailed account of a statistical investigation of the frequency of metastases in the bone. In 309 cases of car-

cinoma of the stomach bone metastases were formed in 2.5 per cent, in 159 cases of carcinoma of the uterus in 3.03 per cent, in 101 cases of carcinoma of the esophagus in 6.9 per cent, in 57 cases of carcinoma of the rectum in 10.5 per cent, in 63 cases of carcinoma of the breast in 52.3 per cent, and in 24 cases of carcinoma of the prostate in 66 per cent. The difference between 3.03 per cent of skeletal metastases in carcinoma of the uterus and 66 per cent in carcinoma of the prostate is very striking, and cannot be explained by mechanical differences. The topographic relationships between the uterus and the skeleton on one hand, and the prostate and the skeleton on the other are practically identical. So is the vascularisation of the two organs, and it is as easy for the cancer cells from the uterus as from the prostate to find lodgment in the bone marrow.

The opinion of Lubarsch[6] that the difference in the relative sizes of the cancer cells and of the minute blood vessels of an organ influence the formation of metastases, is also hardly tenable. The bone-marrow contains blood vessels of a sufficiently large caliber to admit the lodgment of a cancer cell of any size.

The only plausible explanation of this difference between tumors of certain organs seems to be that while both the cells of the uterine cancer and the carcinoma of the prostate find lodgment in the marrow, the latter cells find there a better soil for development, than do cells of the uterine cancer. A very striking instance indicating the existence of a peculiar affinity between the cancer cells and bone tissue was presented recently by Schmorl. He found in a series of cases of carcinoma, metastases, not only in the bones of the skeleton, but in bone tissue abnormally developed in the cartilage of the larynx and trachea, and in one case a metastasis developed in a bone plaque on the wall of the aorta. In the latter case no metastases were formed in any organ with the exception of the bones of the skeleton and of the bone tissue in the wall of the aorta. It is inconceivable that cancer cells should have been able to reach the small bits of bone tissue of the aortic plaque more easily than the parenchymatous organs.

Thus the probabilities are strongly in favor of the conception

that tumor emboli are transported everywhere with the same ease and find lodgment in any part or organ of the body. The further growth, on the other hand, of the small transported group of cancer cells and its development into a large metastatic tumor depends upon a correct metabolic affinity between the cancer cells, on one hand, and the organ cells, the soil as it were, on the other.

The writer[7] has shown in a series of investigations, that the development and growth of inoculable malignant tumors of the lower animals depends upon a local interaction between tumor cells and the somatic cells of an organism. In certain instances the parenchymatous cells of a normal organ may inhibit the growth of the tumor, and this inhibition ceases when the parenchymatous cells become degenerated through a certain injury to the organ. On the other hand the writer[8] has shown that when the cell of a normal organ offers no adequate resistance to the growth of the tumor, then the proliferating tumor cells injure, mechanically or chemically, the former cells. This injury evidently precedes the death of the organ cells and their replacement by tumor cells.

Bone tissue, in view of its characteristic morphological differences from all other tissues, presents a very favorable object for the minute study of the changes taking place in the soil under the influence of tumor growth. In view of the further fact that such a study may help to elucidate not only the pathogenesis of metastasis formation but even the pathogenesis of cancer itself, a microscopical study was undertaken of the skeletal metastases of the cases reported here. The technique adopted in the present investigation was the following. Bone tissue containing the metastases was hardened in 5 per cent formalin and then decalcified in a 5 per cent aqueous solution of nitric acid. The latter solution was changed daily for four days. Further the tissue was washed for twenty-four hours in running water, hardened in 80 per cent and 95 per cent alcohol, imbedded in celloidin or paraffin and stained in hematoxylin-eosin, and by Van Gieson's method.

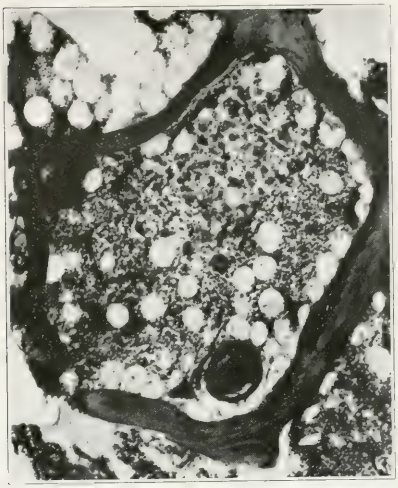

FIG. 3. Microphotograph of a bone-marrow space, showing a small island of cancer cells. (Low magnification.)

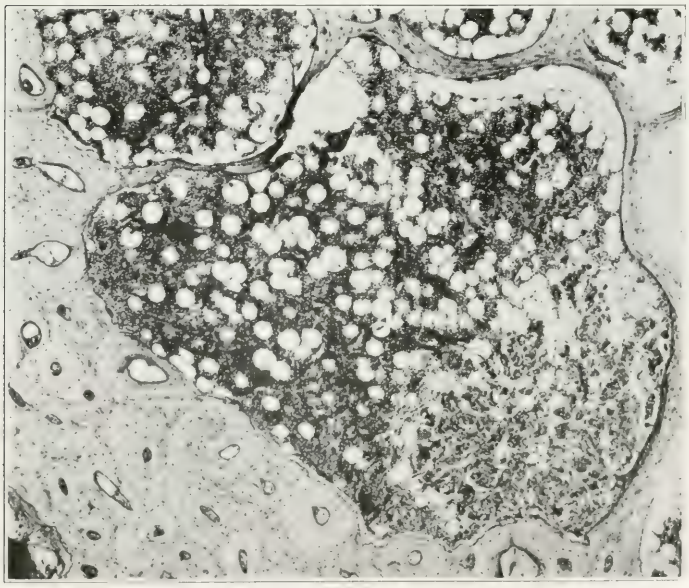

FIG. 4. Microphotograph of a bone-marrow space with a small nodule of carcinoma. (Low magnification.)

The microscopic study of the material brought out several points of importance.

The metastasis begins its development within the marrow, and when the group of cancer cells is small, the surrounding bone-marrow appears quite normal. Figs. 3 and 4 present microphotographs of two such small cancer nodules within a bone-marrow space. Von Recklinghausen claims that the development of the metastasis is preceded by a hyperæmia and hæmostasis, due to obstruction of the capillaries by the tumor emboli. In

Fig. 5. Microphotograph, showing abundant new bone formation in the vicinity of an old bridge of compact bone tissue.

the specimens studied by the writer, hæmorrhages and hyperæmia were noted frequently around large tumor masses, in the femur for instance (see Fig. 2), but not around minute metastatic nodules. In the latter instance as stated above no morphological abnormality could be found in the bone-marrow.

As the tumor nodule increases in size it approaches and invades the compact osseous tissue or the compact osseous parti-

tions of the cancellated bone. Then, there begin to appear the characteristic changes in the bone tissue. It is generally accepted that there are two classes of skeletal metastases of carcinoma: *osteoplastic,* in which extensive new bone formation takes place around the metastatic tumor; and *osteoporotic,* in which the changes in the normal tissue surrounding the metastasis consist in extensive destruction of the compact bone. These two distinct conditions can be easily differentiated on the gross inspec-

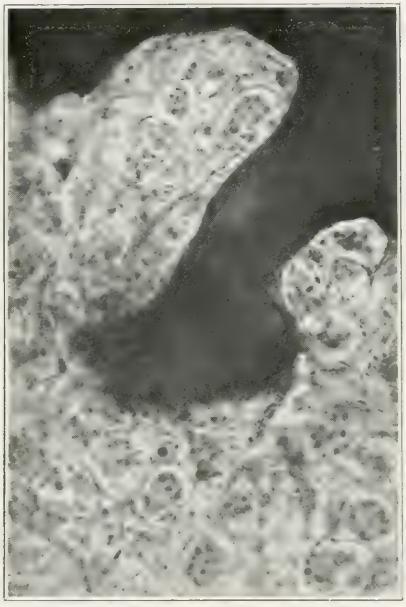

Fig. 6. Microphotograph, showing two bone lacunæ filled with carcinoma cells. (Low magnification.)

tion of skeletal metastases. However, the microscopic study of the cases in the present investigation has shown that both conditions are usually present side by side. The differences in the gross appearance are due to the fact that in one case *osteosclerosis* or new bone formation predominates, while in another *osteoporosis* or the destruction of the old bone is mostly in evidence. Fig. 5 shows an extensive new bone formation, while Fig. 6 presents only destruction of the old bone, and both speci-

mens were obtained from different regions of the same metastatic tumor.

The mechanism of the bone destruction in metastases of carcinoma differs from the one observed in inflammatory osteoporosis. Von Recklinghausen first made the observation that the large polynuclear osteoclasts, which destroy the bone in osteoporosis, are very seldom found in the lacunæ of the bone, surrounding a growing metastasis of carcinoma. This fact was confirmed by most of the subsequent investigators. In view of the absence of the large osteoclasts von Recklinghausen presumed that there takes place in this bone a softening by the removal of its inorganic salts and a subsequent absorption without the aid of any cells, a condition similar to the one found in osteomalacia. Apolant,[9] Erbslöh,[10] Askanazy[11] are also of the opinion that osteoporosis in skeletal carcinoma may take place without the aid of special cells. On the other hand, Wolff[12] and Goetsch[13] believe the cancer cells act as osteoclasts and destroy the compact bone and Axhausen[14] maintains that the small elongated mononuclear connective tissue cells frequently found close to the walls of the lacunæ are special osteoclasts derived from the cancer stroma. In the specimens studied by the writer both carcinoma cells and the small connective-tissue stroma cells were found in immediate apposition to the walls of the lacunæ. The carcinoma cells were so frequently the only cellular elements within the lacunæ of the bone, that there cannot be any doubt but that carcinoma cells do act as direct osteoclasts. Fig. 6 shows two lacunæ filled with carcinoma cells. The impression gained by the writer is that the small stroma cells only subsequently invade between the bone and the carcinoma cells. But even if the possibility be admitted that occasionally the stroma cells may act as osteoclasts, it seems quite apparent that the tumor itself by the aid of its formed elements first destroys the bone, and then grows by occupying the space so formed.

While the mechanism of osteoporosis in skeletal metastases is similar to the mechanism of the destruction of any normal tissue surrounding a growing malignant tumor, the osteosclerosis or the

extensive formation of new bone tissue is characteristic only of skeletal metastases.

Von Recklinghausen claims that this extensive new bone formation is due to the hyperæmia described by him and mentioned above. Courvoisier[15] and Kaufmann maintain that a metastasis in the bone is always surrounded by a zone of inflammation, and that this latter causes the osteosclerosis. Askanazy and Assman think that the metastasis at first produces an osteoporosis which is followed by a bone necrosis. The necrotic bone acting as a foreign body causes a new bone formation. Wolff, Courvoisier and Kaufmann are of the opinion that carcinoma cells may

FIG. 7. Drawing showing collagen fibrils emerging from the old bone and uniting with other fibrils.

act directly as osteoblasts and form new bone tissue. Fig. 7 presents a picture very frequently observed by the writer. It shows collagen fibrils formed in abundance from the old bone. These fibrils gradually unite in thick bundles and subsequently form new bone tissue. The latter is clearly formed from the constituent parts of the old bone tissue. On the other hand, as stated above, the writer did not observe any hyperæmia, inflammation or any other abnormality of the bone-marrow at the beginning of the development of the metastases. Neither did

the writer find any necrosis of the bone in any of the specimens examined. It must be concluded in agreement with Axhausen's conceptions that some unknown chemical irritant emanating from the carcinoma cells acts on the old bone tissue and stimulates its proliferation.

Thus two processes always take place side by side in skeletal metastases. On the one hand the tumor destroys the normal bone tissue, on the other hand the remaining osseous tissue proliferates and creates new bone. The latter is quite probably an attempt at self-defense. The new bone tissue may compress and destroy

FIG. 8. *X*-ray plate of the head of the left femur of Case V, showing a double fracture.

the cancer cells. Case V shows such a condition. A pathological fracture occurred in the right femur, which subsequently became firm and clinically seemed healed. Later a second fracture took place below the first. Fig. 8 shows an *x*-ray plate of the bone. Such temporary healing of a pathological fracture within a skeletal metastasis has been described on several occasions.

The minute microscopic study of skeletal metastases gives the clearest evidence of the interaction which always takes place between the normal organ tissue and the cancer cells during the beginning of the development of a metastatic tumor from a transported cancer embolus. Upon the result of this interaction depends the success of metastasis formation.

The subject may be of practical importance. New bone formation begins as stated above by a new formation of connective tissue (collagen fibrils). The formation of connective tissue is always enhanced by radiotherapy and it is thus possible that radiotherapy may aid in the attempt at self-defense mentioned bove. The writer will consider this subject extensively in another place.

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Discussion:

Dr. Wood: It seems possible that the small size of the cells characteristic of many prostatic carcinomata may account for the high percentage of bone metastases in tumors of the prostate. In cases of breast carcinoma with large cells, nodular or skeletal metastases are rarely seen; whereas in scirrhous growths with small cells there may be early and extensive secondary growths. It seems reasonable to assume that the small cells can more readily enter the lymph channels.

GAUCHER SPLENOMEGALY IN A CHILD

FRANK A. EVANS, M.D.

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This paper is a preliminary report on a case of primary splenomegaly of the Gaucher type, associated with symptoms pointing to lesions of the central nervous system. A complete report will be made later in collaboration with Dr. Tilney, who is studying the nervous lesions.

The patient, a boy, four years and eleven months old, son of a well-to-do Austrian Jewish family of Brooklyn, is the second of three children. The family history is entirely negative. On physical examination the father and mother showed nothing of interest in this connection. The other two children, girls aged nine and three years, have never been ill and physical examination revealed nothing of pathological significance, notably, no anemia and no enlargement of the spleen. The mother has had no miscarriages. All three of the mother's pregnancies were uneventful.

The birth of this patient was instrumental. He was breast fed for about a year and thrived normally during this time. After this he showed signs of indisposition and when eighteen months old had an attack of bronchopneumonia. As a child he had many hard falls and his mother noticed that he always bled easily from the nose and mouth. In one of these falls the patient injured his back and subsequently was treated in a hospital as a case of Pott's disease. At this time, when he was two years old, his physicians observed that his spleen was enlarged. When three and one half years old the left hand and foot began to twitch and he fell frequently as before, but now always to the right side. Later both sides and the face became involved in these twitching movements. One year ago a bilateral decompression was done at Mt. Sinai Hospital, after the first of which the patient was temporarily relieved of the symptoms. When

this twitching recurred, however, it was as severe as before, and was subsequently associated with clonic spasms and attacks of grand mal. While at Mt. Sinai Hospital an enlargement of the liver and spleen was again noted; and here also a Wassermann test on blood and spinal fluid was negative. During the next year the nervous symptoms became worse and the day before his death the patient was sent into the Presbyterian Hospital. From the time he came into the hospital his temperature began to rise rapidly, reaching 109° F. the day of his death, which occurred soon after admission. An autopsy was performed three and a half hours post mortem.

AUTOPSY REPORT

The body was fairly well nourished and developed for a boy of five years. The skin and mucous membranes were very pale but showed no rashes or petechiæ and, except for some livor mortis over the back and sacrum, no areas of discoloration. A bloody discharge from the nose and mouth was noted. There was no superficial glandular enlargement but the inguinal glands were palpable, although soft and discrete. There were several ragged scars over the forehead and the scar of a decompression in each temporal region. The pupils were round and equal, the conjunctivæ and scleræ clear.

On opening the chest a thymus extending 6 cm. below the episternal notch and weighing 12 gm. was exposed. The lungs crepitated throughout but on section showed small red patches in the region of the bronchi. The bronchi were injected and full of bloody mucus. The glands at the hilum were slightly enlarged and pigmented. The heart was normal except for small red patches in the epicardium over the body of the left ventricle, the remains of old petechial hæmorrhages.

The liver was large, weighed 600 gm., and extended 1.5 cm. below the costal margin in the right mid-clavicular line. It was soft and flabby and flattened out on the hand. On section the parenchyma bulged above the line of the cut surface. The color was a pale yellowish-brown. The lobulation was very indistinct.

The spleen weighed 350 gm. It was reddish in color and in one area over the posterior surface showed some fibrinous adhesions. The normal shape of the spleen was maintained. On section the parenchyma was firm, and little material could be scraped from the surface. It was predominantly a pale red color but was mottled with darker red pin-point areas. The trabeculæ were not prominent. The usual appearance of the Malpighian corpuscles could not be made out.

The intestines showed hyperplasia of the lymphoid tissue in the terminal portion of the ileum.

The mesenteric and retroperitoneal lymph nodes were enlarged, varying in size from those just palpable to others one cm. or more in diameter. They were discrete and soft. On section they were a pinkish-gray color of even distribution, except in a few where the reddish tinge was denser in the center.

The bone marrow from the upper third of the femur was moist and dark red in color.

Further than these findings, except for changes in the central nervous system which will be included in the subsequent report, there were no pathological changes.

MICROSCOPICAL EXAMINATION

The lungs showed an intense bronchitis with hæmorrhage in the lung tissue surrounding the bronchioles.

The spleen presented a remarkable picture. The parenchyma was made up of small groups of large cells, surrounded by a thin connective-elastic tissue capsule, and separated from each other by small amounts of normal looking splenic pulp. Some of the groups of cells were densely packed, but in other places they were not so crowded and lay free, together with a few red blood cells, in what could in these places be seen to be venous sinuses. Some of these sinuses appeared to be lined with swollen endothelial cells and others by cells similar to those free in the lumen. The supporting tissue around these sinuses and groups of cells was scanty but everywhere present. The pulp between the distended sinuses was not abundant but appeared to be made up of the

elements normally encountered. The Malpighian corpuscles were widely separated but normal in size, and at the center of each were many of the same large cells that distended the venous sinuses. The usual lymphocytes were somewhat scanty in the center and many of them were fragmented. Surrounding each Malpighian corpuscle was a small zone of hæmorrhage, which gave to the organ its mottled gross appearance. The trabeculæ were about as usually seen. The lymph glands showed many of the same type of large cells that distended the venous sinuses of

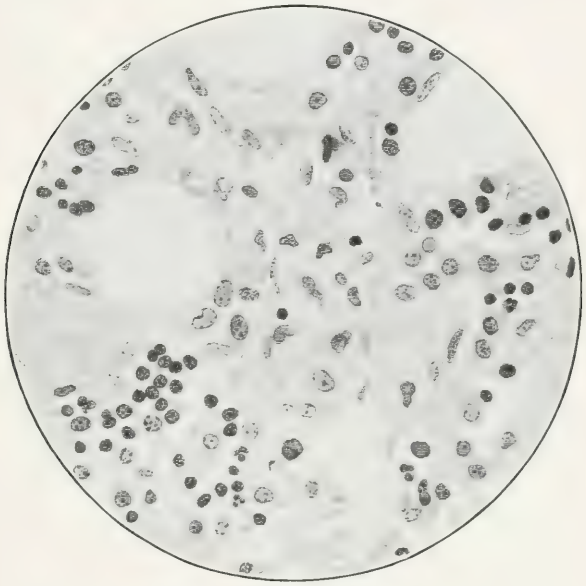

FIG. 1. Bone marrow, showing large cells, with reticulated protoplasm.

the spleen. These were grouped in long cords or small masses for the most part, but some were isolated. The larger groups of these cells were commonly surrounded by a delicate connective and elastic tissue strand, and the long columns were frequently bounded on each side by blood vessels. Lymphoid cells were necessarily reduced in number, but still very numerous. The germinal centers contained many of these large cells and, as in the Malpighian corpuscles of the spleen, the lymphocytes were

often fragmented. The mediastinal lymph glands and those at the hilum of the lung showed the same cells as were found in the retroperitoneal and mesenteric glands. It was interesting to note that none of these large cells in the mediastinal glands contained anthracotic pigment, although other cells in the same section did.

The lymphoid tissue in the terminal portion of the ileum

FIG. 2. Liver, showing a group of large cells between the liver cells.

showed changes similar to those described for the lymph glands. The bone marrow showed, richly scattered throughout its substance, many of these pathological cells, singly and in groups. Although many of them were lying on strands of reticular tissue, many were not, and here as elsewhere the relation of these cells to any specific element was not clear.

The liver presented a lesion difficult of interpretation with the ordinary stains thus far used. The normal-looking liver cells were swollen. Beside these, scattered everywhere throughout the section were smaller, shrunken-looking cells, more faintly staining than those identified as liver cells. They might not be, however, recognized as the same pathological cell that was present in the spleen, but it seems probable that this change was part of the same process, not so frankly manifested here as in the spleen and blood-forming organs.

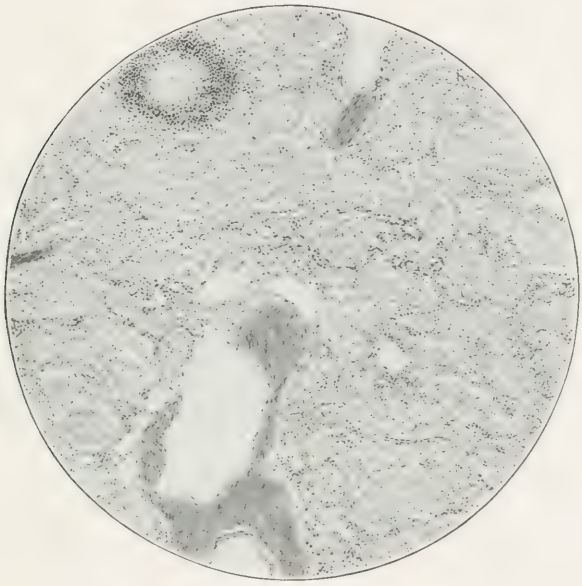

FIG. 3. Spleen.

None of the organs involved in the pathological process contained any iron pigment. The other organs of the body were examined with negative results and will not be mentioned by name, except to say that the enlarged thymus did not show any pathological cells.

The pathological cells in all of the organs except the liver were essentially the same. They were large, varying from 20 to 40 microns in diameter, often round, but more commonly oval or

elongated. Sometimes they were drawn out into long strands. The nucleus was small (7 microns) and densely staining in most cells, but in a few was larger and clearer. It was always eccentrically placed. The protoplasm was abundant, faintly staining, and contained no vacuoles. There was often, however, an irregular area, usually on one side of the nucleus and extending up to it which stained very faintly or not at all. The protoplasm contained delicate strands running in the long axis of the oval or elongated cells. In those that were round these strands were

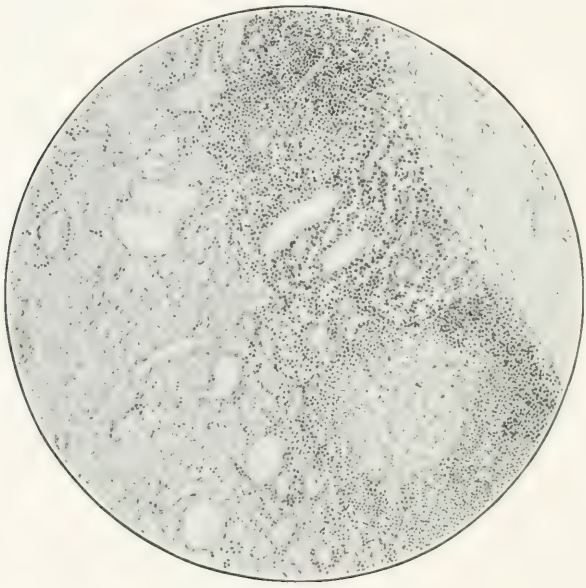

FIG. 4. Lymph Gland.

difficult to trace but seemed to take no particular course. Where the cells were crowded together in long sheets and were drawn out, this longitudinal striation of their protoplasm gave a characteristic appearance to the section. Occasionally these cells were crowded together into what appeared to be a syncytial sheet. The formation of giant cells was very rare. These cells contained no lipoid that could be demonstrated by osmic acid, Sudan III, Scharlach R, or the myelin sheath stain of Wright.

They did not contain an oxydase ferment when tested with the indophenol blue reaction, and no mitochondria could be seen in them after staining with Heidenhain's hematoxylin or with acid fuchsin and methyl green after the method of Bensley.[1] As to the direct relation of these cells to other elements, little can be said with certainty. Van Gieson and Mallory connective tissue, and Weigert's elastic tissue stains showed that, in all the organs involved in this process, these pathological cells were often in close relation to reticular elements, and, where densely packed in the sinuses of the spleen, were lying directly on the reticulum. In some of the sinuses, where not so densely packed, they were separated from the reticular tissue elements by a layer of swollen cells that strongly suggested endothelium.

INTERPRETATION OF THE AUTOPSY FINDINGS

In view of the history and pathological findings we believe that this is an example of primary splenomegaly of the Gaucher type. It differed from the typical picture as established by Bovaird,[2] Risel[3] and Mandlebaum,[4] in several respects. No other members of the family are at present suffering from a similar affection; there were no discolorations of the skin or lesions of the conjunctivæ, and the liver was not so extensively or frankly involved in the process as in the classical type. The case is of further interest in that it was associated with symptoms pointing to a lesion of the central nervous system. As yet we have not determined whether this was closely associated with, or entirely independent of the Gaucher's disease.[1]

It is noteworthy that the same question as that of the origin and relationship of these pathological cells, arises also in regard to the so-called reticulo-endothelial cells of Goldman,[5] Evans,[6] Kiyono,[7] and others, which are specifically stained intra vitam by pyrrol blue, trypan blue, lithium carmine, etc. These cells have

[1] If these pathological cells of Gaucher's disease can be shown to be the same as the vitally staining cells, the association of nervous lesions with this disease might be expected. McCurdy and Evans (*Berl. klin. Woch.*, 1912, xlix, 1695) have shown that the vitally-staining histogenous macrophages can, and under some conditions do, proliferate in the central nervous system.

practically the same distribution in the body as the pathological cells in Gaucher's disease. It would be of interest in cases of Gaucher's disease to study carefully the milky patches of the omentum which are made up almost entirely of these vitally staining cells. In this case, unfortunately, no sections were taken from the milky patches, but gross examination of the omentum showed no abnormality. On the other hand, in this case the mediastinal lymph glands showed the vitally staining cells loaded with anthracotic pigment side by side with the pathological cells, none of which contained any pigment. Until Gaucher's disease is reproduced in animals capable of being vitally stained, or until a case of typhoid fever superadded to Gaucher's disease becomes available, no positive statement can be made about the identity of these pathological cells with the normal vitally-staining cells of the body. However, a study of this case has led me to the belief that the pathological cells are not of endothelial origin exclusively, or of reticular origin, or of lymphoid character arising in the germinal centers of the lymph glands and Malpighian corpuscles alone; but that they are identical with the vitally-staining reticulo-endothelial cells, and that knowledge acquired as to the latter cells will apply to the pathological cells of Gaucher's splenomegaly. The agent exciting the great proliferation of these cells in Gaucher's disease is not known, but the reticulo-endothelial cells proliferate moderately under certain known circumstances,[8] and one author[9] gets a proliferation of similar cells by the injection of lipoids. I realize that this statement will meet opposition, and admit that a careful differentiation between the large cells of lipoidemia[9,10,11] and those of Gaucher's disease must be made. But I feel that careful metabolic studies on cases of Gaucher's disease might be of value in throwing light, not only on the etiology of this disease, but also on the physiological activity of that large class of tissue cells, the histogenous macrophages.

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Discussion:

Dr. F. S. Mandlebaum: This case of Dr. Evans interests me particularly because the liver apparently showed no involvement. If this be a true case of Gaucher's disease, and Dr. Evans's presentation leads me to believe that it is, it would be the first case reported without liver involvement. It is often difficult to demonstrate the large cells in liver sections and Dr. Evans should not despair of finding them. The phosphomolybdic acid hematoxylin stain of Mallory will often bring them out when other methods fail. It is interesting that this child was the only affected member of the family, but I know other instances where this is also true. The absence of pigmentation of the skin as well as the absence of the conjunctival lesion might be expected in so young a subject.

It should be emphasized that in true Gaucher's disease no lipoids are found in the cells by microchemical methods. The peculiar substance which is present in the large cells is not soluble in ether or alcohol, but is rendered more prominent by these reagents. A careful study of the morphology of the cell, however, is sufficient to distinguish Gaucher's disease from the lipoidemia cases. The cells in the cases of diabetes associated with lipoidemia, as well as the cells in Anitschkow's experimental animals fed on cholesterin, have a more or less rounded contour, and the vacuoles from which lipoids may be extracted are always round. In Gaucher's disease, on the other hand, the cells show a peculiar streaked or wrinkled appearance of the cytoplasm, and round vacuoles are absent. We have stated definitely, therefore, that the diagnosis of Gaucher's disease may be excluded in any case which bears a resemblance histologically to Gaucher's disease and in which lipoids can be demonstrated in the large cells by microchemical methods.

We agree with Dr. Evans that the large cells are derived from the reticuloendothelial apparatus of the hemopoietic organs, and that in all probability they are identical with the cells involved in lipoidemia, in vital staining, and in Anitschkow's cholesterin fed animals. The substance in the cells, however, differs in each instance and depends upon the underlying etiological factors. The results of our chemical analyses lead us to believe that in Gaucher's disease some metabolic disturbance with the production of a complex protein substance, probably in combination with the phosphatids, must be considered.

Dr. Lambert: I have studied sections from Dr. Evans's case and I do not think he intended to say that the liver was not affected. The liver was indeed very much altered. There were queer, pale areas in which the cells did not resemble normal liver cells, but they were different also from the large cells of the spleen and lymph nodes and we hesitated to interpret them as Gaucher cells.

A CASE OF LYMPHOSARCOMA OF THE SIGMOID

A. L. GOODMAN, M.D.

The patient, a child of four years, was admitted to the German Hospital, Feb. 24, 1916, complaining of chronic constipation and bleeding from the rectum, accompanied with extreme pallor and marked weakness. The child had been somewhat constipated for about four months, and more recently had had severe abdominal cramps before defecation. For three weeks there had been frequent attacks of tenesmus, accompanied by the passage of pure blood—at one time as much as half a pint. When the severe symptoms began the mother noticed a hard lump in the child's abdomen, which seemed to change its position from day to day and to grow gradually larger.

On admission the child did not seem acutely ill. She was extremely pale but fairly well nourished. The cervical, axillary and inguinal nodes were palpable and the tonsils markedly hypertrophied. There were signs of mitral insufficiency and hypertrophy of the heart. The abdomen was scaphoid in type.

On inspection a tumor was seen occupying the right umbilical and inguinal region. On palpation this could be felt as a hard, irregular, nodular mass which seemed very superficial. It lay

parallel to Poupart's ligament and measured 13.5 cm. in length and 5.5 cm. in its broadest diameter, being twice as broad above as below. It could be displaced beyond the median line and above the umbilicus and moved with respiration. The tumor was roughly sausage-shaped. It was rather doughy to the touch and not tender. There was no tenderness or rigidity of the abdominal wall.

Physical examination showed nothing else of importance. The Wassermann and von Pirquet tests were negative.

The blood count was as follows: Hemoglobin 55 per cent.; white blood cells 1,160; polymorphonuclears 62 per cent.; lymphocytes 38 per cent. The blood pressure was 110. The urine contained a faint trace of albumen, many pus cells, a few red blood cells, but no casts. Examination of the feces showed pure blood, blood clots, scant fecal material and no ova.

On admission to the hospital the abdominal tumor lay in the right iliac region. With care it could be moved from one side of the abdomen to the other. Two days later, February 26, the tumor lay in a transverse position across the abdomen, corresponding to the transverse colon. On February 28, it had moved across to the left side of the abdomen just below the splenic flexure of the colon.

An *x*-ray examination revealed a distinct obstruction in the large intestine, which apparently was in the distal portion of the transverse colon.

The differential diagnosis was interesting. The history was of a rather acute illness of three weeks' duration. At the onset she was seized with sudden abdominal pain, so severe that she lay on the floor and screamed with pain. Shortly afterward the child passed blood from the rectum. The blood was dark in color and clotted, which would indicate an origin high up in the intestine. The sudden appearance of the tumor and its sausage-shaped character, would lead one to consider the possibility of an intussusception of the intestine. Against the diagnosis were the painlessness of the tumor, the absence of vomiting, and the duration of the swelling as well as the general glandular enlargement

which spoke more in favor of a new growth. The negative von Pirquet and absence of signs of a general tuberculosis, excluded the possibility of a tuberculous condition in the omentum. The constant loss of blood, the increasing pallor, and the gradual increase in size of the swelling made operative interference seem advisable.

A laparotomy was performed on February 28. A tumor of the sigmoid was found, about 6 inches in length, and three inches in width, with metastatic involvement of lymph nodes in the mesentery. The mass was removed and the two ends of the gut were joined together by means of a Murphy button. On March 4 the child died with symptoms of general asthenia.

The pathologist's report is as follows:

Gross Examination: The specimen consists of a portion of the sigmoid with an incorporated tumor mass. The entire specimen measures 33 cm. in length. At either end is a segment of approximately normal gut, one measuring 13 cm. in length, the other about 1 cm. The remainder of the intestinal wall is enormously, but somewhat irregularly thickened, so that it forms an ovoid tumor mass about 6.5 cm. in diameter at its widest part. Through this mass the lumen of the intestine runs eccentrically. The thickening of the walls extends unevenly around the whole circumference, in the thinnest portions measuring about 1 cm.; in the thickest, about 3 cm. On section through the growth, the mucosa of the intestine appears to be replaced by a white nodular tumor mass which in one area is extensively ulcerated. The cut surface of the gut wall is of chalky white color resembling lymphoid tissue. The outer surface is irregularly nodular and rather soft. In the mesentery are found some enlarged lymph nodes.

Microscopical Examination: A section through the tumor shows the original structure of the intestinal wall to be practically obliterated by a diffuse sarcomatous infiltration, composed of closely packed small round cells about the size of lymphocytes. Only in a few regions are found traces of the smooth muscle and of the original mucous membrane. Externally there is a thin

capsule of fibrous tissue, evidently peritoneum, but this also shows some infiltration with tumor cells in most regions. The blood vessels are very scanty and consist for the most part of thin-walled capillaries unsupported by connective tissue. Nevertheless, there are no marked areas of necrosis in the sections examined. At its margins the tumor invades the stroma of the mucous membranes and appears gradually to merge with the normal lymphoid tissue of the mucosa. Mitotic figures are exceedingly abundant.

Anatomical Diagnosis: Lymphosarcoma of the large intestine.

One finding seemed to explain the passing of dark clotted blood in this case. In the center of the tumor was a small cavity with three deep ulcerations in its wall from which bleeding took place. The blood apparently accumulated in this cavity and clotted, and as the opening from the lower end of the tumor mass into the gut was so small that one could hardly force the little finger through it, these clots may have remained in the cavity for some time before being passed. Such a possibility should be taken into account when considering the significance of clotted blood in the stools.

In reviewing the literature of intestinal lymphosarcoma it is interesting to note that in most of the cases reported, the onset of the symptoms was acute. The reason for this is apparently that with the growth of the tumor the lumen of the gut is gradually restricted until it becomes so small that it is impossible for the fecal matter to pass. This obstruction gives rise to symptoms which simulate acute or chronic intussusception of the intestine, from which condition it must always be differentiated.

These cases, while not extremely rare, are infrequent enough to warrant an individual report.

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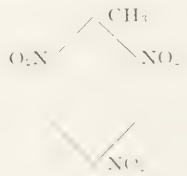
TRI–NITRO–TOLUENE POISONING

H. S. MARTLAND, M.D.

(*From the Pathological Laboratory of the City Hospital, Newark, N. J.*)

Tri-nitro-toluene has the following graphic formula:



It is known in the munition industry as TNT, and is the high explosive of the present European War.

It has from fifteen to twenty times the explosive strength of nitro-glycerine. To explode TNT, it is necessary to bring it up to a certain heat and then produce concussion. Heat alone causes it to burn like gum-camphor, concussion alone will not explode it. A shell filled with TNT is usually exploded by placing in the nose of the shell a detonator containing another high explosive such as fulminate of mercury.

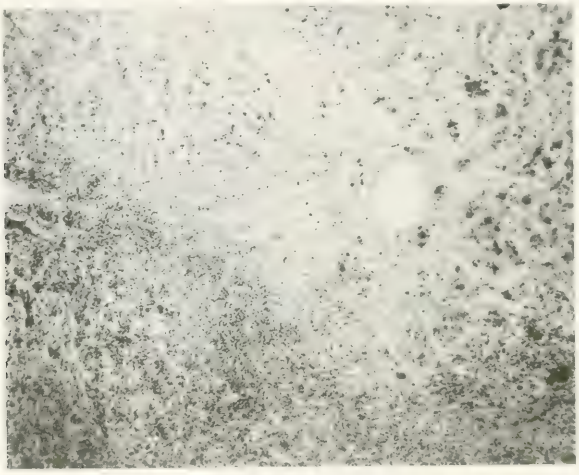


FIG. 1. Tri-nitro-toluene Poisoning. Section of liver showing extensive and diffuse necrosis and fatty degeneration, with perilobular lymphocytic and polynuclear infiltration.

In the manufacture of TNT there is little danger of industrial poisoning, since the procedure takes place in closed retorts, etc., with, consequently, no exposure of the workmen to the fumes. In the filling of high explosive shells it is necessary to heat the flaked TNT above its melting point, to about 94° C., and after letting it cool slightly, to pour the molten resin-like substance into the steel shell and then allow it to solidify. It is during these procedures that the workmen are exposed to the heavy fumes emitted by the hot TNT. It is afterwards necessary to chip and scrape off the solidified explosive which has collected around the top of the shell. An accurate core must also be drilled

into the solidified TNT to make room for the detonator. During these processes, especially if proper protective machinery is not in use, the workmen are exposed to the yellow dust.

It is very probable that TNT poisoning has been known in Germany for some time. Reports of it however have not been made public. There has been little about it in the French or English literature until quite recently. In 1915, in England, a committee was appointed under the chairmanship of Sir George Newman to make a report to the Ministry of Munitions on industrial diseases occurring in munition factories and workshops. In a part of their report, Memorandum VIII, the symptoms of TNT poisoning are described and measures for minimizing the risks are outlined.

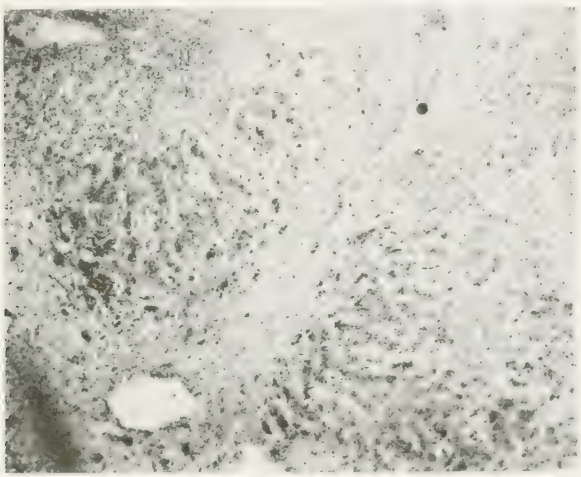


FIG. 2. Tri-nitro-toluene Poisoning. Section of liver showing extensive necrosis with bile-duct proliferation around periphery of lobule.

In the early part of 1916, Spilsbury (1) demonstrated before the Section of Pathology of the Royal Society of Medicine four livers presenting the following types of toxic hepatitis:

1. *Tetra-chlor-ethane Poisoning.*—Tetra-chlor-ethane is used to dissolve acetic cellulose in preparing a water-proof varnish for coating the wings of aeroplanes; it has been the cause of

several fatal industrial poisonings in aeroplane factories, being commonly known as "dope" poisoning.

The liver showed irregular fibrosis and cellular destruction, with very marked regeneration in one part, and almost complete absence of fat.

2. *Di-nitro-benzol Poisoning.*—The liver showed irregular fibrosis, very little degeneration of cells, much bile-duct proliferation and no fat. This was obviously an example of a very chronic form of toxic hepatitis.

3. *Tri-nitro-toluene Poisoning.*—The liver showed marked reduction in size, irregular fibrosis and very advanced fatty degeneration.

4. *Acute Yellow Atrophy.*—The liver was characterized particularly by absence of fat in the liver cells and marked fatty degeneration of the endothelial cells.

In August, 1916, Drs. Livingstone-Learmonth and Cunningham (2) described the symptoms and treatment of TNT poisoning among women workers in some of the large munition plants in England. This is the first comprehensive description that we know of. The symptoms are mainly due to the inhalation and ingestion of TNT dust. Exposure to the fumes alone might give the same effects, with this exception, however, that the irritating effects on the respiratory tract would be less.

The symptoms observed were divided into irritative effects due to action of the poison on the skin and mucous membrane, and toxic symptoms due to the degenerative hepatitis.

The former include nasal irritation with occasional epistaxis, lacrymation and smarting of the eyes, a sensation of constriction in the throat, and dry cough with yellow bitter sputum. Patients complained of a bitter taste in the mouth, of spasmodic griping pains in the abdomen, of acid eructations and often of vomiting. Early in the intoxication there was obstinate constipation followed by diarrhœa with tenesmus. The skin showed rashes of a cheiropompholix or eczematous type over the face and extremities, most prominent where sweating was greatest.

The toxic symptoms included nausea, vomiting, anorexia,

jaundice, mental depression, blurred vision, and transient peripheral neuritis. The poisoning terminated in delirium or sometimes in coma or in convulsions.

REPORT OF CASE

The case observed was that of a white man, 24 years of age, who had always enjoyed good health. In April he started work in a munition plant devoted to the filling of shells with TNT.

He was employed in the mixing and stirring rooms, and his work consisted in dumping the flaked TNT into steam kettles stirring it as it melted, and filling shells with the molten substance. There was no TNT dust in the room.

When he had been at this work for seven weeks, he complained to the factory doctor of nausea and vomiting, slight abdominal pains and general weakness. Except for jaundice and tenderness in the epigastrium the physical examination revealed nothing.

He laid off work but grew gradually worse, and two weeks later was removed to a hospital, markedly jaundiced, vomiting persistently, prostrated and slightly delirious. There was no fever. He died in coma five days after admission.

The striking features of the case were the obstinate constipation, abdominal cramps, tenderness in the liver region, nausea and vomiting, prostration, and progressively deepening jaundice, the low muttering delirium, anuria, and terminal coma. Similar symptoms are common in delayed chloroform poisoning, phosphorus poisoning, acute yellow atrophy, Weil's disease, and certain forms of fatal malaria. In all these diseases the symptoms suggest hepatic failure due to extensive destruction of liver cells.

The autopsy was performed three days after death. As the body had been embalmed it would be of little value to give the entire autopsy protocol. The anatomical diagnosis was: Toxic hepatitis with extensive destruction of the liver parenchyma, toxic degenerative tubular nephritis; icterus; cloudy swelling of heart muscle; increased blood destruction in the spleen; and secondary anemia showing considerable polychromatophilia.

A striking feature was the intense jaundice. The skin of the entire body was a deep chrome yellow and all of the viscera were bile-tinged. The anatomical changes of chief interest were those in the liver, which I will describe in detail.

Gross Examination.—On opening the abdomen the free edge of the liver presents two fingers' breadth above the free costal margin. The organ is small and contracted. The serosa is smooth, glistening and free. On section the cut surface as a whole is grayish in color except for blotchy red areas where the embalming is imperfect. The region of the efferent veins is generally light red in color, soft in consistency and somewhat collapsed. The periphery of the liver lobule is greenish gray, hard and firm.

Microscopic Examination.—There is cloudy swelling of liver cells with extreme fatty degeneration followed by necrosis and autolysis of liver cells leading to extensive destruction of liver parenchyma. This destruction is so extensive that liver cells in their normal cord-like arrangement are no longer seen, except occasionally in areas around and near a portal space. In the middle and inner parts of the liver lobule around the efferent veins the liver cells are no longer recognizable. Around the periphery of the liver lobule there is extensive infiltration, chiefly of small lymphocytes with occasional wandering cells, but few polynuclears. The bile-ducts at the periphery of the liver lobules show extensive regeneration and proliferation. Throughout the liver tissue there are areas showing fine, greenish, granular pigment; in places this pigment is so abundant as to form cord-like masses, the so-called bile thrombi. There are numerous scattered areas showing hemorrhage, the tissue being filled with erythrocytes. Sections stained for fat show marked fatty degeneration of the liver cells, the few left being often filled with small fine irregular fat globules. The necrosis seen in the liver is so extensive and diffuse that it is quite impossible to ascribe to it any zonal distribution, but there is some evidence that it is more severe in the region of the efferent veins, the best preserved liver cells being usually in the region of the periportal space.

The picture is that of a toxic hepatitis, characterized by extensive cloudy swelling, fatty degeneration and necrosis of the liver cells, most marked in the region of the efferent veins but not distinctly zonal in character; lymphocytic perilobular infiltration; bile-duct hyperplasia; jaundice, and areas of extensive hemorrhage. The damage is beyond any known possibility of liver-cell regeneration.

References:

1. Spilsbury, B. H. Demonstration of a Series of Livers Affected by Toxic Hepatitis. *Lancet,* 1916, i, 999.
2. Livingstone-Learmonth, Agnes, and Cunningham, Barbara M. The Effects of Tri-nitro-toluene on Women Workers. *Lancet,* 1916, 261.

THE CLASSIFICATION OF DIPHTHEROID BACILLI BY FERMENTATION REACTIONS

MALCOLM McBURNEY, M.D.

(*From the Department of Bacteriology, College of Physicians and Surgeons, New York*)

I wish to give merely a preliminary report on a series of sugar fermentations with about 60 different strains of diphtheroid bacilli. The work was begun in this laboratory by Mr. F. Eberson, who isolated most of the strains, and turned them over to me only when he left this country to take a position abroad. Considerable work has been done by others in the last few years on this class of organisms and I shall cite briefly some of the results obtained and theories advanced, especially in regard to the etiology of Hodgkin's disease.

In 1914 Bunting and Yates described the production of what appeared to be Hodgkin's disease in monkeys by the injection into the lymphatic glands of cultures of diphtheroid bacilli which were obtained from the glands of patients suffering from this disease. They were able to obtain the organism again from the glands of the monkeys inoculated and to transfer the disease from one monkey to another.

Billings and Rosenow in 1913 reported improvement in cases of Hodgkin's disease following the injection of diphtheroid vaccines.

Bloomfield, however, in 1915 obtained cultures of diphtheroids from lymph nodes not only in cases of Hodgkin's but in a large variety of other diseases involving these glands and in a few cases from normal nodes. Moreover, all agglutination and complement fixation tests with the serum of the Hodgkin's patients were negative and large doses of diphtheroid vaccine caused no reaction.

Fox confirmed these results and also showed that the fermentation reactions of different strains of diphtheroids varied greatly. Similar results were also obtained by Torrey. The strains with which I have worked have been obtained from almost all parts of the body, and have all apparently belonged to the indefinite diphtheroid group. For my sugar reactions I used saccharose, maltose, dextrose, dextrin, lactose and mannit in serum water with Andrade's reagent. My tests were repeated always twice and usually three or more times, and transfers were always made from tubes showing no acid formation to prove that growth had taken place. Frequent microscopical examinations of smears were made to rule out the possibility of contamination.

The sources from which my strains were obtained are as follows:

Lymph nodes in Hodgkin's disease,
Lymph nodes in pseudo-leukæmia,
Lymph nodes in chronic leukæmia,
Oral pus pocket,
Anal pus pocket,
Ascitic fluid,
Blood of indefinite febrile case,
Hypertrophied tonsils,
Bone marrow,
Urine,
Eye,
Nose,
Skin,
Brain in case of meningitis,
Urine in case of gonorrhœa,
Preiz Nocard bacillus from a horse,
Preiz Nocard bacillus from a sheep,
Preiz Nocard bacillus from a calf,
Acne pustule,
Ear, otitis media,
Appendix,
Blood in pneumonia,
Bile, cholecystitis.

Among the eleven strains obtained from cases of Hodgkin's disease are several, kindly sent to us by Bunting and Yates, and by Rosenow.

In the fermentation tests the strains divide themselves into eight different ferment groups. A large number form no acid, a few ferment all the sugars, and the remainder can be divided according to their sugar reactions into six different groups. In seven of these groups are one or more strains obtained from Hodgkin's glands. In the same way strains from the eye, the nose, etc., do not fall into one but into several groups. Several strains of the Preiz Nocard bacillus, however, all fall in the same group. It is now some time since these reactions were completed and I intend to repeat a number of them in order to see if they remain constant after protracted growth of the organisms on culture media and can be relied upon as a means of differentiation.

A number of Hodgkin's strains have been injected into rabbits, living and in large amounts and have absolutely no effect upon the animals. So far, I have been unable to stimulate the formation of agglutinins in rabbits by the injection of these organisms.

It seems very unlikely that any of the strains with which I have worked can have any etiological relationship to Hodgkin's although it is quite probable, as suggested by Bloomfield, that the organisms grow more readily in tissues already the seat of disease.

Discussion:

Dr. Krumwiede: There is one statement that I should like to comment upon, namely, that the negative fermentation tubes gave abundant growth on transfer. This method of checking negative results is frequently mentioned in recent bacteriological literature. It is, however, no indication that the bacterium cannot ferment the carbohydrate. I had, recently, a large series of strains from an outbreak of septic sore throat. In Hiss serum-water medium containing salicin, not one would ferment the salicin, though transfers gave abundant growth. If peptone were added, however, fermentation promptly occurred. Wolbach recently reported a series of streptococcus strains, many of which failed to ferment lactose in serum-water, although milk was coagulated. He checked the results by the presence of growth and drew certain conclusions from the results. Evidently the coagulation of the milk was due to acid production from the milk lactose, the milk being a medium more favorable for the development of the fermentative action of the cocci, and not to some other factor.

Dr. Lambert: Did you get the organism from all the cases studied? How many Hodgkin's glands did you investigate, and what percentage of cases with Hodgkin's disease showed this organism?

Dr. McBurney: A pretty large percentage. One gland had a large abscess, secondarily infected with staphylococci, but the diphtheroid was there and was very easily plated out. We had the same result with a gland from leukæmia.

Dr. Libman: What method did you use for the studies of acid production?

Dr. McBurney: We used the Hiss serum-water medium with Andrade's reagent.

Dr. Libman: Some years ago Drs. Celler, Sophian and myself were doing some work on streptococci, and we pointed out that the Hiss medium has not an active value for the study of acid production because bacteria do not grow readily in it. The results were better if peptone was added. We found that the best medium for the testing of acid production for bacteria is a fluid medium containing non-heated serum and carbohydrate; the next best was agar containing unheated serum and carbohydrate; the third best was a fluid medium containing carbohydrate alone, and the poorest medium was agar containing carbohydrate alone.

We came to the same conclusion as Dr. McBurney as regards the studies that had been reported in Hodgkin's disease. There is no doubt that organisms are found quite often in diseased lymph nodes and other non-healthy tissues. Apparently such organisms get used to living in such a tissue and then when cultures of them are injected into animals they have an affinity for similar tissue. In such instances we are dealing with the condition of latent microbism.

Dr. McBurney: In regard to the criticism of acid reactions, we tried to get differentiation between various strains; we did not try to get as many strains to form acid as possible. My object was merely to show that various strains are not identical.

Dr. Ewing: In any discussion of the bacteriology of Hodgkin's disease mention should be made of the work of Torrey, which has shown that several types of diphtheroid microörganisms are commonly present in the lymph nodes of this disease, none of which appears to have definite pathogenic action or deserve to be accepted as the exciting factor in the malady. He and Bloomfield have also shown that another microörganism, called *B. lymphophilus,* is constantly present in the nodes, while rarely found elsewhere, but again the indications are not satisfactory that it is the pathogenic agent in the disease.

Dr. MacCallum: I have not seen the results of Bunting and Yates. I would like to ask if they have seen anything in experimental animals which resembles the sharp, specific character of the involvement in Hodgkin's glands? If not, it seems to me that the analogy rests on an insufficient basis.

Dr. Libman: We found the organism in the tuberculous lymph nodes. There was one case in Boston in which it was found in acute leukæmia. Dr. Olitsky found the complement fixation negative in Hodgkin's disease. He never got complement fixation in human beings, though he did in animals.

Dr. McBurney: Bunting and Yates in their cases got enlargement of the lymph nodes by repeated injections in monkeys. A large number had secondary infections besides the diphtheroid bacillus. The sinuses after injection often did not heal, and their descriptions of the lesions do not correspond closely to Hodgkin's disease.

STUDIES ON THE PARATYPHOID GROUP AND ANTIGENIC AND CULTURAL DIFFERENCES IN STRAINS OF B. TYPHOSUS

HARRY WEISS

(From the Department of Bacteriology of the College of Physicians and Surgeons, New York.)

Early in my study of the paratyphoid and typhoid groups, cultural and antigenic variations among the strains within these groups were observed. Variations from the fixed types have been noted by others but no classification in the individual groups has to my knowledge been published. This preliminary report on the work attempts to show that such a classification may be made.

PARATYPHOID A

The cultural and antigenic investigation of twelve strains, isolated from human cases, has shown that paratyphoid A strains differ from one another in both respects. Tests were made for their ability to ferment dextrose, mannit, maltose, lactose, saccharose, xylose, rhamnose and inosite. Mr. J. S. Rice, of the Roosevelt Hospital laboratory, was my co-worker on inosite fermentation. The results of the fermentation, agglutination, and agglutinin-absorption tests are shown in Table I. "A" indicates acid formation in twenty-four hours; "G" indicates gas formation in twenty-four hours. Delayed reactions are indicated by small letters. The figures in parenthesis indicate the titer of the respective sera with the strain against which the animal was immunized. Two other paratyphoid B sera, not shown in the table, were also employed. They gave results parallel with those recorded.

Nine of the strains of paratyphoid A studied reacted identically in the fermentation and agglutination tests. Three other strains showed both cultural and antigenic differences from the typical strains and from each other. The use of xylose was sug-

gested by Harding and Ostenberg[1] and recently extended by Krumwiede, Pratt and Kohn[2] who in a recent issue of the *Journal of Medical Research* have established the fact that the non-fermentation of xylose differentiates paratyphoid A and a part of the group which we speak of as heterogeneous paratyphoid from the other members of the paratyphoid-enteritidis group.

TABLE I

Paratyphoid A

	Agglutination				Fermentation							
	Typhi murium Serum (1–16,000)	Paratyphoid B Serum (1–16,000)	Paratyphoid A Serum (1–16,000)	Agglutinin Absorption	Dextrose	Mannit	Maltose	Rhamnose	Inosite	Xylose	Lactose	Saccharose
9 strains	0	1–400 or below	1–8,000 to 1–1,600	+	AG	AG	AG	Ag	0	0*	0	0
Strain No. 167	0	1–2,000	1–8,000	+	Ag	Ag	Ag	Ag	0	0	0	0
Strain No. 322	0	1–2,000	1–8,000	+	AG	AG	Ag	Ag	0	0	0	0
Strain No. 168	0	1–400 or below	1–2,000	0	AG	AG	AG	Ag	0	0	0	0

PARATYPHOID B

The twenty-five paratyphoid B strains studied were isolated from human cases. The majority differed culturally from the A strains in that they produced gas on xylose and on inosite. All of these strains with one exception (No. 169) agglutinated well in paratyphoid B anti-serum. So far as tested they agglutinated also in a typhi murium serum. All failed, however, to absorb the agglutinins from the latter. The absorption tests carried out with two paratyphoid B sera brought out interesting differences between the strains, which are shown in Table II. One group absorbed the agglutinins from both sera. A second absorbed them from the "army" serum but not from the "Seeman"; and conversely, one strain was negative with the "army" serum and

* It is interesting to note that eight strains of paratyphoid A developed a very small amount of gas in xylose broth, though no acid was formed. On account of the absence of acid we have tabulated the fermentation as negative.

positive with the "Seeman." A fourth group failed to absorb the agglutinins from either. On the basis of the plus or minus reactions—that is tests which showed a very feeble agglutination with the homologous strain after absorption—three other intermediate antigenic types may be distinguished.

None of these strains agglutinated in an antiserum for paratyphoid A above the zone of group reactions.

TABLE II

Paratyphoid B

	Agglutinin Absorption			Fermentation								
	Paratyphoid B, Army	Paratyphoid B, Seeman	Typhi murium	Dextrose	Mannit	Maltose	Rhamnose	Inosite	Xylose	Lactose	Saccharose
12 strains	+	+	0	AG	AG	AG[1]	AG	G	AG	0	0
1 strain	+	+	0	AG	AG	AG	AG	0	AG	0	0
2 strains	+	0	0	AG	AG	AG	AG	G	AG	0	0
1 strain	+	0	0	AG	AG	AG	AG	G	A	0	0
2 strains	+	±	0	AG	AG[2]	AG	AG	G	AG	0	0
1 strain (No. 1691)	0	+	0	AG	AG	AG	AG	G	AG	0	0
4 strains	0	0	0	AG	AG[3]	Ag	AG[4]	0	AG[5]	0	0
1 strain	±	0	0	AG	AG	AG	AG	0	AG	0	0
1 strain	±	±	0	AG	AG	AG	AG	G	AG	0	0

[1] One strain fermented maltose only after forty-eight hours.
[2] One strain fermented mannit only after forty-eight hours.
[3] One strain gave delayed fermentation on mannit.
[4] One strain gave acid only.
[5] One strain gave fermented xylose only after forty-eight hours.

B. TYPHI MURIUM

Five strains of bacilli from mouse typhoid were studied with the results shown in Table III. There are at least two groups represented, one which gives positive agglutination and absorption reactions with the serum used, and another which does not. The former may be further subdivided on the basis of the inosite fermentations. The agglutinating group ferments xylose slowly (4–5 days).

TABLE III

B. typhi murium

	Agglutination				Fermentation				
	Paratyphoid A Sera (1–16,000)	Paratyphoid B Sera (3 sera) (1–16,000)	Typhi murium Serum (1–32,000)	Absorption Typhi murium Serum	Dextrose, Mannit and Maltose	Rhamnose	Xylose	Inosite	Lactose and Saccharose
Strain W.....	1–200	4,000–8,000	1–32,000	+	AG	AG	ag	o	o
Strain P & S....	1–200	4,000–8,000	1–32,000	+	AG	AG	ag	G	o
Strain 138 K....	o	+	1–32,000	+	AG	AG	ag	G	o
Strain 13.....	1–40	o	o	o	AG	AG	AG	o	o
Strain K.....	1–100	o	o	o	AG	AG	AG	o	o

B. CHOLERÆ SUIS

Seven strains of *B. choleræ suis* were studied. Variations noted in the fermentation reactions are shown in Table IV. One strain (112 K) produces acid but never gas. All but one strain agglutinated in paratyphoid B serum and also in an antiserum for *B. typhi murium* not shown in the table. They failed, however, to absorb completely the agglutinins from these sera and in this respect differ sharply from the typical members of these other groups. The one strain which failed to agglutinate is evidently a type distinct from the others. The remaining four all showed slight individual differences in the fermentation tests.

TABLE IV

B. choleræ suis

	Agglutination	Fermentation					
	Paratyphoid B Serum (1–16,000)	Dextrose	Mannit	Maltose	Rhamnose	Xylose	Lactose, Saccharose and Inosite
Strain No. 332 K.L.....	o	Ag	AG	Ag	Ag	Ag	o
3 strains.....	1–8,000	Ag	AG	Ag	Ag	Ag	o
Strain No. 112 K.....	1–8,000	A	A	A	A	a	o
Strain No. 334 K.....	1–8,000	Ag	Ag	Ag	a	o	o
Strain No. 120 K.....	1–8,000	Ag	Ag	Ag	A	Ag	o

[1] Twenty-five paratyphoid B strains, six strains of *B. choleræ suis* and two *B. abortus* which were tested, all failed to absorb the agglutinins from this serum.

B. ABORTUS

The two strains of *B. abortus* which we used gave typical cultural and agglutinative reactions. The failure to absorb out typhi murium and paratyphoid B serum showed, however, that they differ antigenically from six of our seven groups of paratyphoid B and from *B. typhi murium.*

HETEROGENEOUS PARATYPHOIDS

The twenty-one strains of paratyphoid included in this group came from a variety of sources, but in no case has proof been found that they are the cause of paratyphoid fever. Several of the cultures were stock laboratory strains which have no history. Isolations from stools were made in cases of nephritis, German measles, paratyphoid B and pyelitis. Several strains were obtained from urine. Two isolations, one from the blood and the other from the urine, were made from a surgical case. Recently I have isolated eight more such strains, one from a paratyphoid A case, one from a dysentery case from which the bacillus Rosen was also isolated, and six from the stool of normal individuals.

Uhlenhuth and Hübener obtained an organism from a case of meat poisoning which was similar to paratyphoid, but did not agglutinate in sera of the fixed types. This organism, which they called paratyphoid C, was agglutinated by the patient's serum and also by an enteritidis serum. Messerschmidt, Babes and Feodorasco, Arzt and Boese, and Sieffert and others have also found these paratyphoid-like types.

Our organisms reacted culturally like typical paratyphoid bacilli, all producing acid and gas very actively on dextrose, mannit and maltose, and being negative in lactose and saccharose They differ from the fixed types in their non-agglutination in paratyphoid A or B sera. Three sera made from strains belonging to this group reacted with the homologous strain only and showed no cross agglutination.

SUMMARY

The fermentation reactions characteristic of the paratyphoid group are the production of gas and acid from dextrose, mannit,

maltose, and rhamnose, and the failure to attack lactose and saccharose. A number of exceptions were noted in this series. One strain produced only acid on dextrose, mannit, and on maltose. On rhamnose three cultures reacted in this atypical manner, producing acid but no gas. Considerable variation was also noted in the speed of gas production and slow fermentation was characteristic of the hog-cholera strains.

On xylose, paratyphoid A is negative, while paratyphoid B, *B. typhi murium*, and *B. choleræ suis* ferment with acid and, with few exceptions, with gas production also. This gives a convenient fermentative differentiation.

On inosite, paratyphoid A, *B. choleræ suis*, and *B. abortus* are negative; while paratyphoid B and *B. typhi murium* each contain a fermenting and a non-fermenting group.

The results of the agglutination reactions are more difficult to summarize. It is evident, however, that ten of our twelve strains of paratyphoid A reacted alike and that thirteen out of twenty-five strains of paratyphoid B reacted alike, as did three out of five strains of *B. typhi murium* and six out of seven strains of *B. choleræ suis*.

In each of these groups, however, are certain strains which show differences in their behavior toward immune sera which set them apart. The reactions suggest that there may be as many as six other antigenic types among our paratyphoid B cultures. A number of the remaining strains we hope to classify more definitely when we obtain homologous agglutinating sera.

B. TYPHOSUS

In my investigation of the *B. typhosus* I studied the cultural and antigenic properties of thirty-one strains coming from different sources.

Cultural Reactions.—I can at this time report the reactions upon eight fermentable substances: dextrose, mannit, maltose, xylose, lactose, saccharose, rhamnose and inosite. In every case where an atypical reaction occurred the test was repeated and not accepted unless checked, and the possibility of contamination eliminated by plating out the culture.

The most marked cultural variation occurred in the xylose fermentation. Of the thirty-one strains, twenty-two produced acid in twenty-four hours, three acted slowly fermenting sufficiently for a positive reading on the third day, and six remained negative after prolonged incubation. One of these is the Rawlings strain. Four of them are atypical antigenically as will appear later.

The strains were uniformly negative on rhamnose and inosite. That the non-fermentation of rhamnose differentiates *B. typhosus* from all the classified members of the paratyphoid-enteritidis group was pointed out by Uhlenhuth. Twenty strains reacted typically producing prompt acid fermentation in dextrose, mannit and maltose and failing to ferment the other sugars. Five other strains showed delayed fermentation in one or another of the sugars as indicated in Table V.

TABLE V

Cultural Variations in Strains of B. typhosus

No. of Strains	Dextrose	Mannit	Maltose	Lactose	Saccharose	Xylose	Rhamnose	Inosite	Litmus Milk	Antigenic Group
20	A	A	A	o	o	A	o	o		Ia. Ib. IIIa
1	A	a	A	o	o	A	o	o	alk. 5 d.	II
1	A	a	A	o	o	A	o	o	acid	IIIb
1	a	A	a	o	o	a	o	o		Ib
6	A	A	A	o	o	o	o	o		Ib. IIc. IIIa
2	A	A	A	o	o	a	o	o		Ib

Agglutination Reactions.—The agglutinations were carried out with nineteen typhoid strains on four antityphoid sera before the more recently acquired organisms had been isolated or received. Two strains failed to agglutinate in one of the sera used and a third did not agglutinate in any. The remaining organisms could be further divided into two classes on the basis of the strength of their reaction.

Absorption Reactions.—It is difficult to make a classification on the basis of the intensity of the agglutination reactions though the differences brought out were extremely suggestive. For a

further study of these differences, absorption tests were resorted to which were carried out in the following way. The sera were diluted to one eighth of the highest dilution in which agglutination occurred with the homologous strain. Thus, in a serum that had a titer of $\frac{1}{16,000}$ an initial dilution of $\frac{1}{2,000}$ was made. One half c.c. of this was absorbed with an entire slant agar culture of *B. typhosus* suspended in 0.5 c.c. of isotonic salt solution. This was incubated at 37.5 degrees for two to four hours and kept at ice-box temperature for four days. Whenever the supernatant fluid became clear, dry antigen was added and the tube again incubated. The organisms were then centrifuged off, a half c.c. of the supernatant fluid now in a dilution of $\frac{1}{4,000}$ was tested for agglutinins with the homologous strain. This gave a final dilution of $\frac{1}{8,000}$. Wherever there was partial or incomplete absorption, a sharp agglutination occurred.

Thirty strains were tested for their ability to absorb agglutinins from each of these four sera. Nineteen of them absorbed all agglutinins from each of the sera and four other strains were positive on three sera and negative in one. The other seven fell into five different classes as shown in Table VI.

The relationship between the antigenic and cultural characteristics of these strains is indicated in the right-hand column of Table V. Regarding this relationship it would appear that

TABLE VI

Antigenic Variations in Strains of B. typhosus

Division	Sub-grouping	No. of Strains	Agglutination in Typhoid Serum	Absorption Tests			
				Serum R. I.	Serum Rawlings	Serum P. and S.	Serum Novy
I	*a*	1	+	+	+	+	−
	b	10	+	+	+	+	+
II		1	−	−	−	−	−
III	*a*	2	+	−	−	−	−
	b	1	+	−	−	−	+
	c	2	+	+	+	−	−
	d	1	+	+	+	−	+

they are to a large extent independent of one another. Yet there is a tendency for the antigenically atypical organisms to be cul-

turally atypical, and the fact stands out that with one exception, the organisms that were culturally typical belong to one of the two typical antigenic groups.

SUMMARY

Culturally, six of the typhoid strains studied differed from the typical races in failing to ferment xylose. Five other strains fell into four separate classes on the basis of the speed with which they fermented the test substances.

Antigenically, the majority of the strains reacted alike in absorbing the agglutinins from all the sera used. However, in this comparatively small series, eleven strains were found which fell into six different groups when classified by absorption tests.

It is not claimed that these studies have separated typhoid bacilli definitely in six cultural and in seven antigenic groups. They show, however, that cultural variations do occur and that a classification is possible on the basis of the absorption tests. Such a grouping will probably be of importance in typhoid prophylaxis, as a vaccine prepared from a single strain may not give complete protection.

The work will be continued with a larger series of strains in an effort to arrive at a more definite classification.

References

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2. *Jour. Med. Res.*, 1916, xxix, 355.
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A CASE OF POLYPOSIS OF THE SMALL INTESTINE ASSOCIATED WITH CARCINOMA

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The material which forms the basis of this report is the terminal three feet of the ileum, with the exception of two or

three inches next the cæcum, which was removed at operation by Dr. Steedly of Spartanburg, S. C. The patient was a man seventy-four years old. He had suffered from diarrhea and indigestion for 25 or 30 years, and lately, in addition, from chronic obstruction. On palpating the abdominal wall, the affected coils of intestine gave the impression of a solid tumor in the lower umbilical and right inguinal regions. At operation the mesentery was found to be very much thickened, and the affected coils were largely adherent. It was thought at operation that all the affected coils had not been removed.

On gross appearance the intestine presents a thickening of the walls, gradually increasing from above downward. The whole intestinal wall is very firm and rigid. The mucosa of the first eight inches of the specimen appears relatively normal, but rather suddenly the valvulæ conniventes become obliterated and small hard pin-point nodules appear scattered over the surface. Passing down the intestine, very numerous polypoid masses are seen. They average about 4 or 5 mm. in diameter. Some are flat and sessile, others pedunculated. Where the process is farthest advanced there are four or five somewhat longer masses, about 1 cm. across; and 8 cm. from the cæcal end there is a lobulated mass as large as the end of the little finger. The mucosa between the polyps is thick and hard. Nearer the cæcal end the mucosa is so very much thickened that the lumen of the intestine is practically obliterated and is represented merely by a narrow slit. On cross-section it is seen that the muscle coats are greatly thickened, and that scattered throughout the wall and beneath the peritoneum are hard grayish nodules varying in size.

On microscopical examination the sections show that the entire specimen is the seat of a marked chronic enteritis. The polypoid masses, taken from the part where the lesion is least advanced have the structure of adenomata, with the epithelial cells, however, showing evidences of beginning malignancy by the fact that the nuclei are hyperchromatic, and the cells show many vacuoles. These polyps also are the seat of chronic inflammation. Where the lesion is most advanced the sections show a fully developed diffuse adenocarcinoma with nests of car-

cinoma cells in the lymphatic spaces of the submucosa, and in many places the carcinoma has extended through the muscle coats.

To summarize then, this is a case of diffuse polyposis affecting the ileum, associated with chronic enteritis and carcinoma. The fact that it occurs in an individual seventy-four years old is interesting, since the majority of cases reported have been in much younger individuals, from twenty to forty years.

The answer to the ever-recurring question of the relationship of the three processes—enteritis, adenoma and carcinoma—to one another is difficult to determine definitely in this case. The sharp line of advance of the polyps showing beginning malignancy would indicate that the stimulus for the formation of both polyps and carcinoma were the same. Then, as Lubarsch thought possible in his cases, the chronic enteritis could be regarded as being, at least in part, their underlying cause.

SITUS INVERSUS

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The specimen consists of the thoracic and abdominal viscera removed from a female, thirty-two years of age, who died as the result of carbon-monoxide poisoning, the gas having been taken with suicidal intent. The specimen was obtained through the courtesy of Dr. Warren, coroner's physician of Newark.

The transposition is complete and absolute. It involves single organs which belong to a certain side or location as heart, liver with gall-bladder, stomach, pancreas, spleen, colon and appendix and paired organs as the lungs and kidneys. The whole viscera form a complete mirror-like inversion of the normal condition.

The organs are otherwise, anatomically speaking, normal in appearance and there is no other associated congenital anomaly or fault.

The large vessels going to and from the base of the heart are completely transposed. The arch of the aorta, for instance, turns to the right and the thoracic aorta descends over the right side of the vertebral bodies. The three large arteries arising from the arch are completely transposed. There is no other circulatory anomaly present, which is important when we consider that one of the two romantic theories regarding the etiology of this condition is based upon anomalies of the circulation of the early main current of blood.

The brain is quite normal in appearence from the anatomical standpoint. We are unable to ascertain whether the woman was a right- or left-handed individual nor do we know whether she was one of a twin pregnancy.

EXPERIMENTAL STUDIES OF CHOLESTEROL METABOLISM

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It is at present generally accepted that cholesterol, which is apparently a primary cell constituent of animals, is not synthesized from simpler substances by the animal organism, but is taken in the food, and has its ultimate origin in the phytosterols, a group of closely related substances occurring in plants. It must be said, however, that conclusive evidence against the animal synthesis has never been brought—that there is at least a chance that as our knowledge of the synthetic capabilities of animal cells gradually widens, it may be found that under some circumstances a synthesis of cholesterol may also take place. On the other hand, it has been shown definitely that it is absorbed from the intestine, since feeding on a cholesterol-rich diet leads to an increased amount in the blood and organs. From this point on, nothing is known of its intermediate metabolism. Lifschutz has brought considerable evidence to show that it is partially oxidized

in the body and that the cholic acid radicle of the bile salts is produced from it. This has not as yet been confirmed and others hold that it is not destroyed in the body, but is excreted in the bile, perhaps (as Rothschild believes), as a result of a specific activity of the Kupffer cells. Reabsorption from the intestine follows; the degree depending, perhaps, upon the needs of the body and the amount supplied in the food.

It is evident that we have here only the barest outline of the process, quite lacking in all details. In order to be able to understand the disturbances in the normal metabolism of cholesterol which seem to occur in cholelithiasis, fevers, pernicious anemia, and other conditions, it is essential to have more detailed information on the subject and with that end in view, the work which is to be described was undertaken.

The question of the mechanism of the absorption is logically the first to be considered. From the similarity of cholesterol and its fatty acid esters with the neutral fats, absorption might be expected to take place from the intestine by way of the lymphatics. This was readily found to be the case in dogs. Chyle from a thoracic duct fistula after feeding eggs, or pure cholesterol dissolved in cotton-seed oil, showed a much higher cholesterol content than a specimen from the same dog before feeding. Tables I and II present two typical cases.

TABLE I

Fistula Completed at 1 p.m.

Chyle		Free Cholesterol		Cholesterol Esters		F : T[2]
Time Collected	Amount	Amount		Amount		
p.m.	*gm.*	*gm.*	*per cent.*	*gm.*	*per cent.*	
1.00	13.4	0.0049	0.036	0.0088	0.066	35.29
5.30[1]	15.2	0.0060	0.039	0.0100	0.066	37.02
7.30	9.6	0.0059	0.062	0.0115	0.119	33.91
8.30	10.0	0.0088	0.088	0.0207	0.207	29.83
9.30	9.8	0.0111	0.113	0.0270	0.276	28.32

[1] Time of feeding.

[2] Figures in this column indicate the percentage of the total cholesterol which is free.

TABLE II

Fistula Completed at 1.30 p.m.

Chyle		Free Cholesterol		Cholesterol Esters.		F : T
Time Collected	Amount	Amount		Amount		
p.m.	*gm.*	*gm.*	*per cent.*	*gm.*	*per cent.*	
4.30[1]	10.7	0.0026	0.024	0.0045	0.042	35.38
6.45	9.8	0.0093	0.095	0.0133	0.136	41.13
8.45	9.7	0.0065	0.067	0.0100	0.103	30.41
a.m.						
12.30	10.0	0.0067	0.067	0.0106	0.106	38.73

[1] Time of feeding.

It will be noted that both the free and combined cholesterol increase and that the proportion between the two remains nearly constant. This is true when free cholesterol is fed as well as when cholesterol oleate is given, and apparently represents an adjustment of the proportion of the free and combined to that normal to the chyle and also to the blood plasma.

Since nothing has been known of the interchange between the free and combined forms of cholesterol in the body, this change during absorption seems to offer a particularly fortunate opportunity for more detailed study. There are conceivably three agencies to be considered, namely the lumen of the gastro-intestinal tract, its mucosa, and the mesenteric lymph nodes, and each of these has in turn been investigated.

TABLE III

		Free	Combined	F : T
		per cent.	*per cent.*	
Dec. 30.	Biliary fistula made.			
Jan. 1				
11.30 a.m.	Thoracic duct fistula completed. Chyle taken	0.021	0.028	43
1.30 p.m.	Chyle taken. Fed small piece of meat.			
1.45 "	Fed four raw eggs.			
5.00 "	Chyle taken, very milky	0.056	0.038	60
6.00 "	" " " "	0.058	0.037	61

By excluding experimentally certain digestive juices from acting upon cholesterol fed to dogs, and studying the changes in

the chyle during absorption, certain information has been gained. Normal absorption was obtained by injecting a solution of cholesterol in cotton-seed oil directly into the duodenum, thus ruling out gastric digestion. The latter is therefore probably not essential for its absorption. After ligating and severing the common bile-duct and making a gall bladder fistula, a greatly modified absorption was obtained in several dogs. The following table will illustrate this change.

The total increase is seen to be much less than in normal absorption. In addition, over 60 per cent. of the total cholesterol is free, as opposed to 30 to 40 per cent. ordinarily found. Evidently the bile plays rather an important part in cholesterol absorption normally.

In a number of dogs, too, the pancreatic ducts were ligated and cut, leaving the organ itself in situ, with blood supply as nearly intact as possible. Absorption of cholesterol through the chyle in such dogs was practically nil, pointing to the importance of the pancreatic enzymes in the process.

TABLE IV

	Titration	Cholesterol		E. T
		Free	Combined	
	cc.	*gm.*	*gm.*	
Free cholesterol 0.02 gm., fresh	3.8	0.0212	0.0006	97
" " 0.02 " incubated	14.8	0.0104	0.0102	50
Cholesterol oleate 0.04 gm., fresh	3.5	0.0062	0.0058	52
" " 0.04 " incubated	17.0	0.0032	0.0100	24

Certain experiments were undertaken to see whether there could be demonstrated an action in vitro by the enzymes of the pancreas and intestinal mucosa, alone and in the presence of bile, upon cholesterol in the presence of fatty acids, and upon cholesterol oleate. Although in all such experiments neutral fat present was readily split as evidenced by a titration of the fatty acids formed, no splitting of cholesterol oleate was ever observed. On the other hand, when a suspension of pancreas in dilute glycerol was used, free cholesterol was found after incubation to have become to a marked degree converted into cholesterol esters. In

parallel experiments in which intestinal mucosa and a suspension of lymph nodes were used, in both of which an active lipase was present, no such change took place, and it seems that this may explain, to some degree at least, the necessity for pancreatic juice in the absorption of cholesterol. This esterification is illustrated in Table IV.

In another series of experiments the intestinal mucosa of dogs was carefully washed, removed and analyzed. Several analyses were made on dogs which had received no food for twenty-four hours, and a number on dogs which had been fed cholesterol two or three hours before being killed.

TABLE V

	Duodenum			Jejunum			Ileum		
	Free	Ester	C : T[2]	Free	Ester	C : T[2]	Free	Ester	C : T[2]
	gm.	*gm.*		*gm.*	*gm.*		*gm.*	*gm.*	
24 hrs. fasting	0.0169[1]	None	—						
24 " "	0.0092[1]	0.0006	6.1						
24 " "	0.0131	None	—	0.0098	None		0.0116	0.0006	4.9
Fed three eggs	0.0101	0.0021	17						
Fed 300 cc. of milk with 2 gm. cholesterol in 8 cc. of cottonseed oil	0.0111	0.0017	13						
Fed three eggs	0.0194	0.0061	24	0.0215	0.0064	23	0.0131	0.0014	9.7
Fed two eggs	0.0079	0.0014	15	0.0105	0.0012	10	0.0071	0.0002	2.7

It is evident that there is little combined cholesterol normally present in the intestinal mucosa of dogs, while a considerable amount is found during absorption, when the cells and lymphatics are filled with lipoidal material.

From these observations it seems clear that the esterification of the free cholesterol must take place in the small intestine under the influence of the pancreatic juice, perhaps aided by the bile. The esters are then absorbed as such. No evidence that the mesenteric lymph nodes exert an influence on cholesterol during its absorption could be obtained although the possibility of such

[1] A mixture of duodenum and jejunum mucosa analyzed.

[2] Figures in these columns indicate the percentage of the total cholesterol which is combined in the form of esters.

an influence was not satisfactorily excluded, nor was it determined how the increase in free cholesterol always seen in the chyle after feeding takes place, and is regulated.

From the time that the cholesterol is poured by the chyle into the blood stream, its changes in form and activities are still largely unknown. Only about one fourth to one third of the total cholesterol of the plasma is free, the rest being in the form of fatty acid esters. In the red blood cells, however, there is normally no combined cholesterol, and in practically all the organs the cholesterol is largely free, as is that excreted by the bile. Where the esters of the chyle and the blood plasma are again split has not been determined. Schultz and Cytronberg claim to have found an enzyme (cholesterase) in the red blood cells and in mixtures of the latter with liver pulp which actively split them. These observations have remained without confirmation and in a number of experiments I was able in no case to obtain results at all comparable with theirs. This splitting of cholesterol esters which undoubtedly occurs in the body is perhaps a function of all cells so intimately bound up in the delicately balanced life processes as to be insusceptible of demonstration under the conditions of autolysis. Perhaps experiments can be devised which may be more successful. I hope to be able to report later on this intermediate metabolism of cholesterol.

In regard to the excretion, little can be said beyond the fact that it must be controlled by a very delicately balanced mechanism. Reabsorption of biliary cholesterol doubtless takes place along with cholesterol of the food. There is, however, a certain amount eliminated by the feces at least some of which probably comes from desquamated cells of the mucosa of the colon. In a dog fed on a fixed diet the feces for a five-day period contained about 0.6 gram cholesterol. In a second five-day period, in which 0.3 gram cholesterol per day was added to the food, 0.8 gram was excreted. In a third five-day period, without the addition of any cholesterol to the food, 0.9 gram was excreted, and in a fourth period, in which one gram of cholesterol oleate was added to the food each day, 1.3 grams were excreted. Evidently a very considerable amount in excess of that ordinarily

present in food, can be absorbed, but the complete absorption of all the food—and biliary—cholesterol which would doubtless lead to various forms of pathological infiltrations with arteriosclerosis or cholelithiasis is in some way prevented normally.

Up to the present stage, perhaps, these experimental studies have added little or nothing to our understanding of pathological conditions. Possibly further work along the lines indicated may be more fruitful. There is, however, one statement in the literature to which reference is frequently made, upon which some light may be thrown. This is the belief expressed by Faust and Tallquist that the severe anemia of the pernicious type often occurring in individuals harboring the *Bothriocephalus latus* may be due to cholesterol oleate occurring in the body of the worm. The idea is at first view somewhat surprising since free cholesterol is well known to be an anti-hemolytic substance, and that a simple ester, which indeed occurs in very considerable quantities normally in the plasma, should be so strongly hemolytic and toxic as to cause a severe anemia when present in the rather small amount found in the worm is at least peculiar. When examined more closely their conclusion is found to be based upon the observations that the ether extract of the dried worm was hemolytic; that after saponification, oleic acid was obtained and cholesterol was identified; and further, that no glycerol was present and hence that the original substance must have been cholesterol oleate. When cholesterol oleate was fed to a dog, sodium oleate, a hemolytic soap, was identified in the chyle, but no cholesterol was present. It is argued from these findings that cholesterol oleate is potentially toxic, since it is split in the intestine, the cholesterol excreted and the oleic acid combined with sodium to form a soap which, when absorbed, acts as a hemolytic substance. The objections to such a view are obvious in the light of our present understanding of cholesterol absorption. Manifestly their failure to recognize cholesterol in the chyle was due to an error in technique, due perhaps to the imperfect analytical methods at that time available. Faust later undertook the study of the effect of feeding large quantities, 10 to 20 grams daily, of oleic acid to dogs. In one case a slight decrease in red count,

from 7,000,000 to 5,000,000, increasing again to 6,000,000 resulted in the course of six months, and in the other case no decrease was recognized. The hemoglobin, however, fell considerably in both dogs, while in *Bothriocephalus latus* anemia it is relatively high. This would seem to show pretty conclusively that the oleic acid which might be set free from a small amount of cholesterol oleate is entirely inadequate to account for the severe anemia seen in some cases of bothriocephalus infection. It may be added that in an experiment recently carried out, a dog was fed daily a gram of cholesterol oleate for a week, and then for an equal time received daily intravenous injections of a gram of cholesterol oleate dissolved in a little sesame oil. The red blood count of this animal remained practically constant throughout at 6,500,000, thus pretty well ruling out any severe hemolytic effect by the compound.

Discussion:

Dr. MacCallum: Dr. Müller's work on this most important subject seems to throw the first real light upon the nature of the absorption of cholesterol. Since this substance has come to be recognized as playing such an important part in many diseases as well as in many normal processes, I hope Dr. Müller will continue the work and perhaps later be able to tell more of the modifications which the esters undergo in the tissues.

Dr. Wood: I am glad that the Faust-Tallquist idea has at last been settled. It never seemed to me likely that a mere trace of oleate could have so extensive a hemolytic effect, but much more probable that the cause is a far more toxic substance. It is interesting to see how much work has been done on cholesterol even though the structural formula has by no means been made out. At present much energy has been focussed on the subject and with the more accurate analytic methods which are available at the present, much more will no doubt soon be accomplished.

CARCINOIDS OF THE SMALL INTESTINE AND THEIR RELATIONSHIP TO THE SO-CALLED CARCINOMA OF THE APPENDIX

B. M. VANCE, M.D.

(From the Pathological Department, Bellevue Hospital, Charles Norris, M.D., Director)

The six cases to be presented, consist of four minute primary epithelial tumors of the small intestine and two similar growths of the appendix. These neoplasms are remarkable in that they show a histological picture similar to that of a very malignant carcinoma, but their mode of growth is rather more benign than the microscope would indicate. The name "carcinoid" has been applied to them because of this very peculiarity.

The four specimens from the small intestine were discovered, quite by accident, in autopsies at Bellevue Hospital. The patients were elderly individuals, with ages varying from forty-nine to sixty years. In every case the neoplasm had no influence on the patient's health, and death was due to some other condition.

The tumors were solitary, and in size varied from a wheat grain to a hazelnut. They took their origin from some structure in the submucous layer and were invariably situated on the side of the gut opposite the mesenteric attachment.

No metastatic nodules could be found in other organs.

Case I was a man sixty years of age. The cause of death was coronary sclerosis. A firm, gray white nodule, the size of a wheat grain, was found in the ileum one foot from the ileo-cæcal valve.

Case II was a man of forty-nine years who died from an empyema of the right pleural cavity. There was a firm, white tumor, as large as a bean, in the middle of the small intestine.

Case III was a woman of fifty-eight years. In addition to the carcinoma of the thyroid which caused her death there was a firm, white nodule, the size of a hazelnut, situated in the ileum about a yard from the ileo-cæcal valve.

Case IV, a man forty-nine years of age, died of pulmonary tuberculosis. A small, white nodule, the size of a bean, was found in the small intestine a short distance from the cæcum.

The histological pictures presented by these four different tumors were similar in all essential features, but disclosed a few minor variations. The growth in Case I was composed of short columnar cells, containing large, ovoid, centrally placed nuclei,

Fig. 1. Case I.

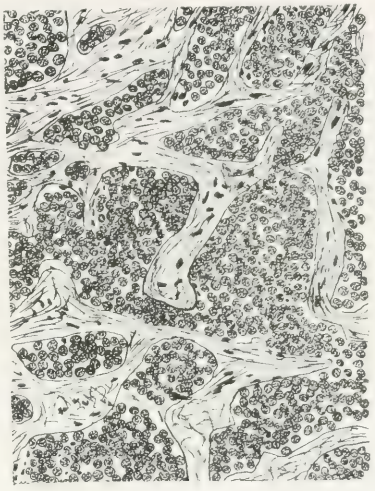

Fig. 2. Case II.

clumped together as solid plugs in alveoli of different sizes. In some of the spaces, the cells near the rim, were arranged in a regular palisade, and there was some attempt at rudimentary gland formation (Fig. 1). The stroma was composed of strands of non-cellular fibrous tissue, containing a very moderate vascular supply. Unfortunately the specimen was not stained by Van Gieson's stain to determine the presence of non-striated muscle in the stroma.

Cases II, III and IV showed the same histological characteristics, and one description will serve for the three. The tumor cells were globular or polyhedral, containing large, spherical, centrally placed nuclei. They were arranged in solid plugs in

alveoli of different sizes and showed no attempt at gland formation (Fig. 2). The stroma was composed of dense, non-cellular fibrous tissue and quite similar to Case I, in this respect. Case II was stained to demonstrate smooth muscle fibers in the stroma, but none could be found.

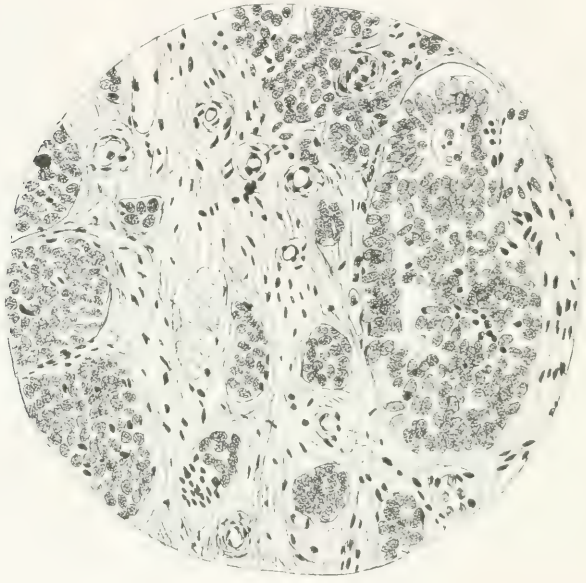

FIG. 3. Case V.

In every instance, the primary site of the tumor was the submucous layer. The growth in Case I showed no infiltration into the surrounding tissue around the nodule. In Cases II, III and IV, the neoplasm had penetrated the muscularis mucosæ, and was destroying the mucous membrane. In Case IV, there was a slow infiltration of the tumor tissue, backward through the muscular layer and sideways into the lymphatics of the submucosa.

The two epithelial tumors of the appendix were very similar to the carcinoids of the ileum, but unlike them were taken from young individuals. Both patients were operated upon for appendicitis and the presence of the neoplasm was not suspected before operation.

Case V. The patient was a woman twenty-three years of age. The appendix was 7 cm. in length, and showed a normal proximal portion, 4.5 cm. long and 6 mm. in diameter. The distal end was a solid, foot-ball shaped, mass of gray white tissue, about 2.5 cm. by 9 mm. by 9 mm. Under the microscope, the distal end was composed of tumor tissue supported by a thick network of fairly vascular connective tissue. The alveoli were large and contained short columnar cells, possessing large, centrally placed ovoid nuclei. The outside rim of cells were in palisade arrangement along the alveolar wall, while the central mass of cells were collected together in solid clumps (Fig. 3). The neoplasm had destroyed the submucous layer and the mucous membrane, and was penetrating the muscular zones. No secondary metastatic tumors were discovered at operation.

Case VI.[1] The appendix removed from a man, eighteen years old, was somewhat larger than that in Case V, but disclosed the same features on gross examination. Microscopically, the tumor consisted of large polyhedral cells, containing large spherical nuclei. They were supported by a thick, connective tissue stroma, and clumped together in solid plugs. The neoplasm had not only destroyed the mucous and submucous layers, but had penetrated to the subperitoneal coat. No metastases, however, could be found in the neighboring lymph nodes or elsewhere.

In both these tumors of the appendix, it is probable that the submucous layer was the point of origin, though this would be difficult to demonstrate in neoplasms of this size. It is evident, however, that in both the cases of the small intestine and the appendix, we are dealing with the same neoplastic type. Most observers now recognize this fact, and identify the so-called carcinoma of the appendix in young people with the carcinoid of the small intestine found in older individuals.

The difference in the ages of the two sets of cases is not easy to explain, but the obvious submucous origin, the slow infiltrative invasion by the neoplastic tissue, and the histological similarities

[1] This case is shown here by permission of Dr. Bulkeley.

between both tumor types declare their close relationship. The so-called carcinoma of the appendix is in reality only a carcinoid, and is not at all like the primary adenomatous, colloid forming, carcinoma found in the intestinal tract.

Cases of genuine primary adeno-carcinoma of the small intestine and appendix, have been described, but these tumors take their origin from the cells of the mucous membrane, and are histologically similar to corresponding tumors in the colon. They are of very rare occurrence, while the submucous carcinoids are relatively common.

A review of the literature shows that the carcinoids of the appendix may occur under a variety of forms. Most of them form a white neoplastic bulb at the tip; others occur as pinhead-sized nodules in the submucous layer. A few grow at the proximal end of the appendix, and others may grow in the middle. Sometimes the whole organ is involved. In the vast majority of cases, the tumor is confined to the appendix, and has been found only occasionally in the neighboring lymph nodes or in other viscera. Histologically, they present very much the same picture as shown by the tumor in Cases V and VI.

The carcinoids of the small intestine are usually accidental findings in old individuals who have come to autopsy from some other cause. These tumors are submucous in origin, and are situated on that side of the gut directly opposite the mesenteric attachment. They may occur as solitary nodules or as multiple primary neoplasms scattered through the intestine. The usual site is the lower end of the ileum, but they may be found in the duodenum, jejunum, and even the large intestine. These tumors show a very slow infiltrative growth, but metastatic tumors in the neighboring lymph nodes and other organs are rare. Under the microscope they present a picture quite similar to the neoplasms in Cases I, II, III and IV.

Many theories have been advanced to explain these carcinoids, but the ones most worthy of consideration have been advanced by Burckhardt and by Oberndorfer. Burckhardt believes that the tumor tissue takes its origin from the normal cells of the intestinal mucous membrane. The cells break off from the

infiltrates the submucous layer very slowly. Some of the cases described by Burckhardt, show structures very similar to the deepest portion of the crypts of Lieberkühn, and the neoplasm intestinal crypts, and on the strength of this he interprets the carcinoid as a tumor of the intestinal mucosa analogous to the basal cell carcinomas of the skin, described by Krompecker.

Oberndorfer takes the view that the carcinoids arise from an embryonic inclusion of enteroblastic cells in the submucous layer of the intestine. The primary multiple occurrence in many cases, the submucous situation of the tumor, and its position on the side of the gut opposite the mesenteric attachment, support this theory, for as Oberndorfer points out, such undoubted congenital malformations of the digestive tract as adenomyomata, and aberrant pancreatic glands, show the same characteristics.

Of the two theories, the one advanced by Oberndorfer is the most plausible, though it does not clear up many points. It is very probable, however, that these carcinoids are small tumor formations derived from congenital inclusions of primitive enteroblastic epithelium. It is also conceivable that the snaring off might have taken place at different periods in the embryonic life of each individual case. The included cells would thus vary in their degree of differentiation which would, perhaps, explain some of the minor histological differences displayed by different carcinoids.

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Discussion:

Dr. Lambert: I saw one of these tumors last year situated in the duodenum in the submucosa. In that one I thought that while the grouping of the cells in solid nests looked like carcinoma, the individual cells did not look like it. They were uniform in size, deeply staining, but did not give an impression of malignancy. I think carcinoid is a good term.

Dr. Wood: The sections are very interesting. The morphology is strikingly like carcinoma of the appendix. We have found the latter in 1 per cent. of cases. Some specimens have tumors of very small extent, but in others the growth involves a very large portion of the lumen and tip. In one case the middle of the appendix was involved. Mitoses are never seen in the cells. The growths seem to be quiescent. I think carcinoids is a good enough name. Still, we know that a basal cell or even a squamous cell epithelioma may stay quietly in the skin for a number of years and then when something starts it up, may become clinically malignant.

HYPOPLASIA OF THE ADRENALS IN ANENCEPHALY

ALWIN M. PAPPENHEIMER

(*From the Department of Pathology of the College of Physicians and Surgeons, New York City*)

The almost regular occurrence of hypoplasia of the adrenals in association with malformations of the brain has been known for many years. At the end of the eighteenth century and the beginning of the nineteenth, when the very minute description of monsters was a favorite pursuit, this association was repeatedly observed. The first observation is attributed by the elder Meckel to Morgagni. In the older references one finds the statement frequently made, that the adrenals in these cases of anencephaly and kindred malformations may be entirely wanting. More recent observers, however, are inclined to be sceptical as to their complete absence. R. Meyer, for example, in studying a large series invariably found the adrenals present, although in some cases the hypoplasia was extreme.

It is natural that much speculation should have arisen as to the cause of this almost constant association. The many theories have, however, led to nothing definite or even plausible. For example, Ilberg has suggested that the failure of the brain and cranial vault to develop may be due to the loss of the hemodynamic action of the adrenal during fœtal life. Were refutation of this idea necessary it would be found in the fact that in many, if not all of the cases, the medullary portion of the gland is present in normal or even excessive amount, and shows no qualitative alterations (Weigert, R. Meyer, Landau and others). Elliott and Armour have, indeed, studied the entire chromaffin tissue in one case, and have found it normally abundant.

Fig. 1. Adrenal and kidney. Natural size.

A new point of interest has arisen in connection with the recent observations of Erwin Thomas, Elliott and Armour, Kern, Landau, Lewis and Pappenheimer and others that a large portion of the adrenal cortex which is so massively developed in the newborn infant is subsequently destined to degenerate. Within the first few weeks of post-natal life the cells composing roughly the inner two thirds of the fœtal cortex disintegrate and gradually disappear, leaving a collapsed vascular framework. We have, therefore, in this portion of the adrenal cortex a large mass of tissue which is functionally significant only in the fœtus. As to what that function may be we are, of course, totally ignorant.

FIG. 2. Section through Adrenal: (*a*) of Full-term Newborn Infant; (*b*) of Anencephalic Infant. Magnification approximately × 10.

It is, however, of great interest to study the behavior of this fœtal zone of the cortex in the hypoplastic adrenals of anencephaly. In the case presented, and in the cases studied by Elliott, Armour and R. Meyer, it is very clear that the reduction in the size of the gland is due, almost wholly, to the diminished size of

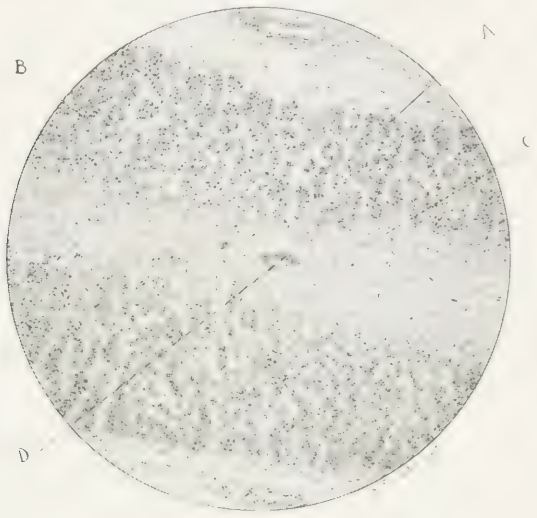

FIG. 3. Section through cortex of adrenal of anencephalic infant. (*a*) Glomerular zone; (*b*) fascicular zone; (*c*) rudimentary fœtal zone; (*d*) nests of chromaffin cells.

this fœtal zone, which is normally doomed to destruction in postnatal life. The zona glomerulosa and the outer portion of the zona radiata—the permanent fraction of the cortex—are normally laid down and have a rich lipoid content in part composed of anisotropic fat. The normally broad inner cortical zone, on the other hand, is represented only by three or four layers of irregularly disposed cells, easily recognized from the outer cortex by their low lipoid content and sharply marked off also from the medullary tissue in the central portion of the gland. The question arises, then, whether in these cases there may not take place in fœtal life an involution of this inner cortical tissue, which in normal infants is deferred to extra-uterine life. This might

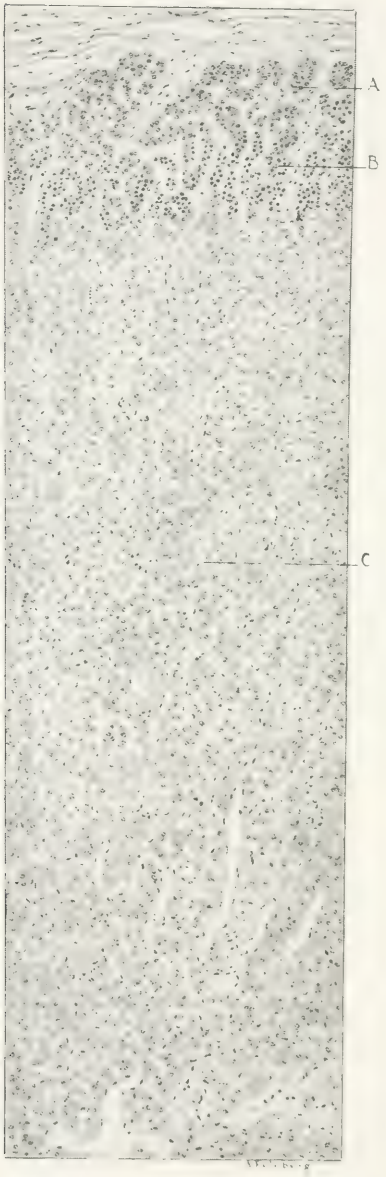

FIG. 4. Section through cortex of adrenal of normal newborn infant. Same magnification as Fig. 3. (*a*) Glomerular zone; (*b*) fascicular zone; (*c*) fœtal zone. No medullary tissue appears in the section.

have seemed probable from the observations which Meyer made upon the adrenals of two anencephalic fœtuses of two and five months in which the adrenal cortex in comparison with that of normal fœtuses of similar age was quantitatively and qualitatively normal. Landau has considered this point, but expresses himself very ambiguously in regard to it, saying in one place that he had never failed to find a "degeneration zone" present in these cases, but in other connections referring to the condition as a true hypoplasia.

From a study of my own case I am inclined to the view that this portion of the cortex has failed to develop rather than that it has undergone a premature degeneration. The individual cells of the thin layer which represents the fœtal zone, are all well preserved. The marked vascularity and the capillary extravasations which accompany the physiological involution of the cortex are wanting in this adrenal, nor does one find the cortical cells delimited from the abundant chromaffin tissue by a fibrous zone, such as ultimately results from the disappearance of the cells and the collapse of the fibro-vascular framework. Blood pigment in this case also is absent, although it is present always in the degenerating post-natal cortex.

The conclusion, therefore, which may be drawn from a study of this case, and from which it would be unwise to generalize, is that the small size of the adrenal is due wholly to the failure of development of the fœtal zone of the cortex; and that the permanent portion of the cortex, as well as the medullary tissue, is present in normal amount. This would suggest that it is this transitory cortical tissue which is in some way correlated with the normal development of the brain.

CANCER OF THE STOMACH IN A MOUSE

SHIGEMITSU ITAMI, M.D.

(From Columbia University, George Crocker Special Research Fund, New York, F. C. Wood, Director)

So far as can be ascertained no tumor of the stomach has been reported in a mouse since the two described by Murray.[1]

FIG. 1. Primary squamous-cell carcinoma of the cardiac portion of the stomach of a mouse.

The mouse about to be described was a white female of unknown age, weighing 24.9 gm. and bearing two adenocarcinomata in the right axilla. These were extirpated on June 5, 1916, and did not recur.

[1] Murray, J. A., Third Sci. Rep., Imperial Cancer Research Fund, London, 1908, p. 69.

In August, it was noticed that the mouse was not eating, and that its weight was decreasing; thus, on August 12 the weight was 25.0 gm., on August 19, 20.1 gm., and on August 26, 19.1 gm. On the 28th of this month, therefore, the animal was killed, and autopsied under aseptic conditions.

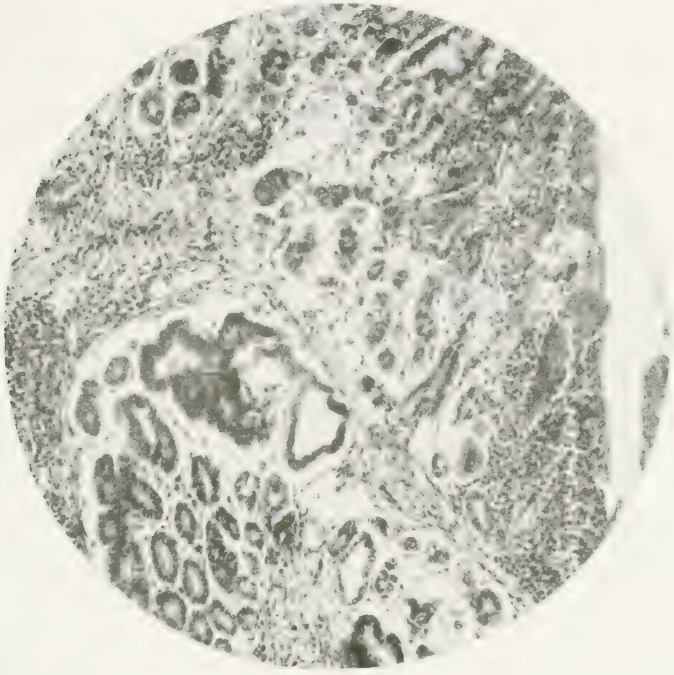

FIG. 2. Invasion of the deeper layers of the stomach by glandular structures, due apparently to irritation caused by the presence of the tumor.

The stomach was found much thickened along the greater curvature, the thickening being diffused over its squamous or cardiac portion. The glandular or pyloric third was not involved. The stomach was not adherent to the adjacent organs, and, except for collapse of the intestine, the other structures of the body appeared to be normal. No macroscopic parasite could be found, nor was any discoverable in the serial sections later examined. A frozen section showed the presence of squamous-cell carcinoma.

A fragment of the tumor was thoroughly washed in normal saline solution and transplanted into forty-eight mice; a larger series was not inoculated because it could be reasonably expected that only the exterior of the growth would be free of micro-organisms, and not much of this could be removed without opening the stomach, a consequence which it was desirable to avoid. Up to the present time no daughter tumor has appeared, though a number of abscesses have developed, as was to be feared.

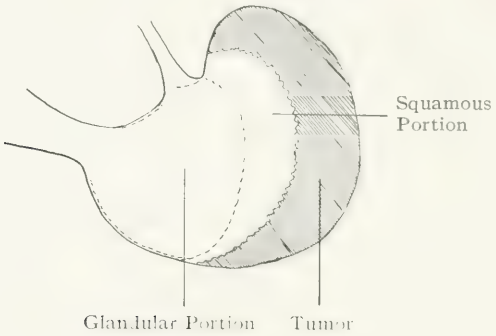

FIG. 3. Tumor of the stomach of a mouse. (Twice natural size.)

Serial paraffin sections, cut longitudinally along the greater curvature, showed a squamous-cell carcinoma situated near the pyloric portion and extending laterally under, and distinctly raising, the remaining normal squamous epithelium. The lower surface of the growth invaded the entire muscular wall, its downward and lateral spread producing an appearance identical with that described by Murray. The tumor was sharply demarcated from the glandular portion of the stomach.

Sections made from the other organs, including the lymph-nodes, revealed no metastases.

Although this mouse had two carcinomata in the mammary region, it is probable that the gastric tumor was not a metastasis. On the contrary, it is much more likely that it arose primarily in the squamous epithelium of the stomach; at any rate, it is directly continuous with this layer, which it closely resembles, and, furthermore, both axillary tumors were purely glandular, containing no suggestion of keratinization.

CARTILAGINOUS AND BONY NODULES IN THE TONSILS

EDWARD G. CARY, M.D.

(From the Pathological Department of St. Luke's Hospital, F. C. Wood, Director)

The occasional occurrence of cartilage in the tonsil has long been known, but it was after Orth's description[1] of two cases of cartilaginous and bony nodules in the tonsil that active interest in the phenomenon developed. Two years after Orth's publication, Deichert[2] reported two new cases in adults and also Orth's second case, a child of two years. Both these authors believed the nodules originated in misplaced embryonal rests of cartilage, coming from the second branchial arch. Deichert summed up the cases in the words, "We have cartilage and bone lying symmetrically in the deep connective tissue of both tonsils in individuals of various ages without especial recognizable pathological changes in the follicular apparatus." In one of his cases, a woman of thirty-five, the presence of a bony nodule 2 cm. in length in the stylohyoid ligament, a long styloid process, and enlarged lesser horn of the hyoid, existing together with cartilage and bone in the tonsil, strengthened his belief in the misplacement idea since all these structures come from the second arch. Furthermore, the nodules were supplied with a well-formed perichondrium and periosteum, and did not show the intimate relation to the surrounding connective tissue that might be expected in cases of metaplasia. The cartilage was hyaline in type and in the child was greatly in excess of bone—bony nodules of a few cells were found. The adults showed a preponderance of bone, formed, he thought, by metaplasia of the cartilage.

Walsham's[3] two cases were adults. One of these showed a preponderance of bony nodules, the other cartilage, and the latter was scattered through the adenoid tissue as well as the capsule and trabeculæ. The bilateral position and absence of hypertrophic changes he thought argued for embryonic origin. Wingrave[4] agreed with Walsham and Deichert that the cartilaginous

nodules might well be of vestigial origin, but because of the multiplicity of the deposits and the frequency of association with morbid changes he felt that the bony nodules were metaplastic.

Töpfer[5] described the transition of connective tissue into cartilage and insisted that the nodules were metaplastic. Pollack[6] also strongly favored the theory of metaplasia for the following

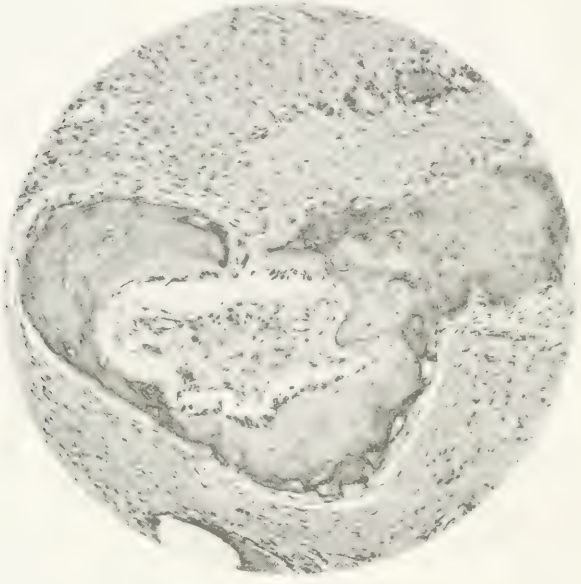

Fig. 1. Bony nodule with bone marrow.

reasons: (1) Inflammatory processes were found in all his cases; (2) the bone occurred in portions showing active inflammation or evidences of inflammation in the past, and was in intimate relation to the connective tissue; (3) cartilage was absent in two cases, and in the third there was bony cartilage, indicating that cartilage is only an intermediate stage; especially since in other areas in the same tonsil the bone had plainly come from the connective tissue. Nösske[7] thought it not improbable that the connective tissue of the tonsil had a special ability for cartilage and bone formation due to developmental characteristics without making an abnormal displacement responsible.

Reitmann[8] in a series of fifty cases, including embryos, infants and adults, found cartilage in the tonsil in 34 per cent. Ruckert[9] found about the same percentage in infants and stated that in all his cases the cartilage was embryonal in character. In a series of 412 cases of all ages from the embryo to advanced old age Lubarsch[10] found the deposits in 16 per cent. Most of these cases were tubercular patients and showed either tubercular lesions of the tonsils or tuberculosis in some other part of the

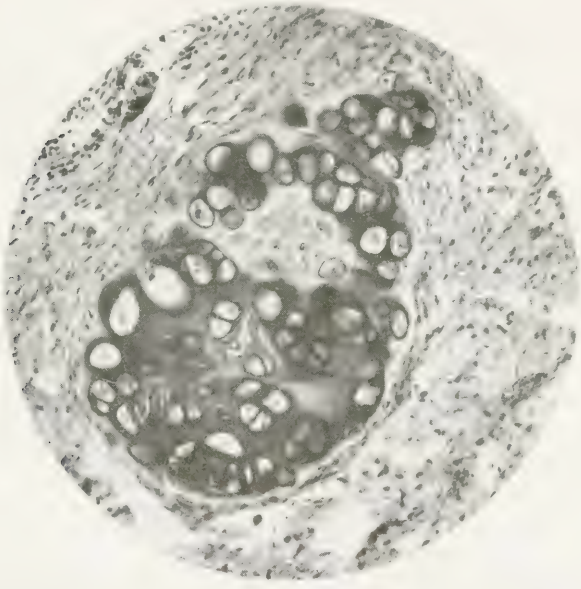

FIG. 2. Cartilaginous nodule with bone in the central portion.

body. The majority, though not all, of Lubarsch's cases showed inflammatory changes about the deposits, and this writer concluded that they arose both from inclusions and from metaplasia of the connective tissue in response to inflammation.

The arguments advanced against the embryonal theory (Töpfer,[5] Pollack,[6] Nösske,[7] Lubarsch[10]) were: (1) The finding of cartilage only in the connective tissue portion of the tonsil and not in the adenoid or follicular part. Walsham[3] and Wingrave,[4] however, reported cartilage in the adenoid tissue. (2) The ap-

pearance of bone and cartilage only in inflamed regions. Deichert,[2] Walsham,[3] Wingrave,[4] Theodore[11] and Grosvenor,[12] however, reported cases in which inflammatory changes were not evident. (3) The observation of continuity of the nodules with bordering connective tissue. On the other hand, Deichert, Walsham, Wingrave, and Theodore describe a definite perichondrium and periosteum surrounding the nodules. (4) The absence of such nodules in children. Nevertheless, Reitmann, Rückert, Lubarsch and Grünwald[13] have demonstrated cartilaginous nodules in embryos, newborn and infants. The bilateral appearance of the deposits was made much of by supporters of the embryonal misplacement theory; but Lubarsch reported unilateral nodules and in these cases the inflammatory process was either present only on the side of the nodules or was most marked on that side.

Neither the metaplastic nor the embryonal theory of origin seems to cover all the cases. Ziegler[14] offers the explanation that besides the fœtal rests, such nodules may develop also in response to inflammation; because, in consequence of the embryonal relation of the tonsillar connective tissue to the branchial arch, it has an especial chondro- and osteoplastic aptitude. Grünwald[15] asserts that the rests come from the fourth, third and second arches, that they ordinarily regress, but that there may be primordial cartilage as well as chondrogenic cells left behind.

The case I have to report is that of a woman of thirty who gave a history of recurrent attacks of tonsillitis. On the cut surface of the tonsils after they were removed could be made out many small gritty spicules that in the microscopic section proved to be cartilaginous and bony structures embedded in the dense capsule of the tonsil and also appearing in the thickened cortical trabeculæ. As evidence of an inflammatory reaction there was swelling of the follicles, partial replacement of the adenoid tissue by connective tissue, infiltration of the cortical trabeculæ by small round cells and plasma cells, and near the crypts were frequent polymorphonuclear leucocytes. In the cap-

sule near the base, the cartilaginous and bony nodules were particularly plentiful. Some of these nodules contained both cartilage and bone, some only cartilage, and a few bone that was not well separated from the surrounding connective tissue. While for the most part the nodules consisting of both bone and cartilage are made up of a central portion of cartilage surrounded by a ring of bone, the reverse condition occurred and the bone was deposited in the central part of a cartilaginous nodule. In places the bone showed a central cavity in which marrow had been formed; and these nodules contained cells corresponding in morphology and position to osteoclasts and osteoblasts. For the most part the bone nodules were supplied with a definite periosteal covering. The cartilage showed a gradual transition to a spindle type of cell and a blending of these with the surrounding connective tissue. The cartilage was hyaline in type with the usual vacuolated cells with reticulated protoplasm, oval and round nuclei, and definite well-formed matrix.

The only point of variance in the above case with those described before is the well-developed central bony deposit surrounded by cartilage. Theodore[11] mentioned as an argument against the inflammatory origin of these nodules the fact that the youngest cartilage cells occurred in the central part of the nodules and that the bony deposits were outside. In the case presented the reverse condition was found.

The theory that best explains the origin of cartilaginous and bony nodules in the tonsil and meets the conditions described by various writers is that, as Ziegler expressed it, we have embryonal rests and an especial chondro- and osteoplastic aptitude of the connective tissue cells in this region.

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Discussion:

Dr. Wood: It is interesting to see how frequently cartilage occurs in the tonsil. I do not think the theory of metaplasia explains the presence of hyaline cartilage. Everybody has tonsillar inflammation to a certain extent, and I doubt that chronic inflammation alone will cause the formation of cartilage. Cartilage occurs near or in the salivary glands, or in regions where, in the embryo, cartilaginous tissue is abundant; so that it has always seemed to me possible that cartilage cells may be left in the tissues and that these may grow if stimulated. Tumors of cartilage occur in the complex regions about the submaxillary and parotid glands and in the region of the bronchial arches where much cartilage tissue disappears early in embryonic life. Some of the cells perhaps remain and start growing later. Formation of bone marrow is common enough where bone occurs. In the aorta and kidney it is found, but I have never seen cartilage formation there.

Dr. MacCallum: I think one ordinarily sees the heteropic development of bone in those places in which necrotic tissue has been preserved for a long time, surrounded by living tissue. There marrow cavities are formed and the bone is laid down by osteoblasts. I have, however, recently seen a similar case in which cartilage was found in considerable quantities in the tonsil.

Dr. Pappenheimer: I have seen islands of cartilage in simple cystic kidney.

Dr. Wood: One might expect that cartilage would be found in congenital cystic kidneys, but my experience is that of Dr. MacCallum. Bone is not infrequently seen in the ovary and in many other sites, but I have never heard of the formation of cartilage in a lymph node, though bone develops often in old necrotic areas in nodes.

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A CASE OF LINITIS PLASTICA

ELI MOSCHCOWITZ, M.D.

The specimen presented is a typical case of linitis plastica, due to scirrhous carcinoma of the submucous coat of the stomach. It is of especial interest because there are no visible changes in the gastric mucosa.

The patient, a man 70 years old, was admitted to Beth Israel Hospital, April 18, 1916. He complained of alternating constipation and diarrhea of three weeks' duration. He had suffered from dull pains in the epigastrium, which were aggravated by food and relieved when the stomach was empty. For the last three days he had vomited all food. He had lost about ten pounds. Physical examination revealed a hard mass deep in the right hypochondriac region, but was otherwise negative. The patient died two weeks after admission. At autopsy the following anatomical diagnosis was made: Linitis plastica; chronic diffuse nephritis; dilatation and hypertrophy of the left ventricle; brown atrophy of the heart muscle; chronic congestion of the viscera.

The stomach was of the typical leather bottle type and unusually small, measuring 12 cm. in the transverse diameter and 8 cm. in the vertical. The peritoneal surface was smooth. The

wall of the stomach was about 1.25 cm. in thickness and fairly uniform throughout. It was very firm and on cut section the submucosa was unusually broad. The mucosa appeared intact throughout revealing not the slightest ulceration or tumor anywhere. It was pale red and covered with a small amount of mucus. The thickening of the stomach wall extended to the first part of the pylorus. There were no enlarged glands.

Numerous microscopic sections taken from various portions of the stomach showed a scirrhous carcinoma involving the submucosa and extending into the muscular coats. No point of origin from the mucous membrane was found. The mucosa showed no infiltration of the growth whatever. There was however no other conceivable origin for the tumor and if sections were taken from many more areas in the stomach wall the primary growth might be found.

PRIMARY ALEUKEMIC SPLENOMEGALY—BANTI'S DISEASE

(Summary)

ELI MOSCHCOWITZ, M.D.

The specimens presented are three spleens; the first two from patients in whom the diagnosis of Banti's disease was made; the third, from a case of splenomegaly associated with polycythemia.

Case I. The patient was a married woman of sixty. For a year she had noticed a steadily growing weakness, and more recently a slight jaundice and swelling of the ankles and feet.

On physical examination she was found emaciated, and profoundly anemic. There were signs of an old tuberculosis at both apices. The liver was slightly enlarged. The spleen extended to the umbilicus. Its edge was irregular and hard. The abdomen contained fluid. The hemoglobin was 35 per cent. and fell to 10–15 per cent. a day or two before death. The red blood cell count was 2,850,000, gradually diminishing to 1,200,000; the leucocyte count 7,000, of which 78 per cent. were polymorphonuclears and 22 per cent. lymphocytes. She died from anemia.

At autopsy the anatomical diagnosis was: Emphysema and healed tuberculosis of the lungs; chronic pleurisy with adhesions, hypertrophy and dilatation of the left ventricle, atheroma of aorta; angioma of the liver, chronic diffuse nephritis and splenomegaly.

The spleen measured 24 × 20 × 15 cm. and weighed 1,165 gm. after fixation. It was densely adherent to the costal wall. The capsule showed the *Zuckerguss* appearance. The pulp was firm but easily indentable, and was deep reddish purple. The connective tissue was enormously increased and the trabeculæ prominent. The Malpighian corpuscles were not visible.

On microscopic examination the spleen showed marked fibrosis, seen as large masses of cellular fibrous tissue which obliterated the sinuses and compressed the veins. The trabeculæ were enormously thickened. The Malpighian bodies were few in number and small and some showed a fibrosis extending out from the central vessel. The pulp was diminished in cellular content and deeply engorged with red blood cells. Many of the vessels in the trabeculæ showed thickened walls. The sinuses were normal in size. The splenic veins in the pedicle showed a marked endophlebitis with plaques of lime and bone in the walls. Sections of both spleen and vessels were stained by the method of Levaditi and no treponema pallidum was found. The liver showed no cirrhosis.

Case II. The second specimen is from a woman forty-seven years old who gave a history of hemorrhages from the mouth and bowel. She complained also of weakness and loss of weight. These symptoms had lasted five weeks. She had suffered a similar attack twelve years before. When admitted to the hospital the patient was pale and emaciated. Her spleen, which extended to umbilicus, was hard and very irregular. The liver was not enlarged. There was ascites and nine to ten liters of fluid were repeatedly removed. Examination of the blood showed: hemoglobin, 50 per cent.; red blood cells 3,460,000; white blood cells, 14,200, of which 85 per cent. were polymorphonuclears, 12 per cent. lymphocytes, and 3 per cent. eosinophiles.

An autopsy revealed healed tuberculosis of the bronchial lymph nodes, brown atrophy of heart, atheroma of aorta and cardiac valves, chronic parenchymatous nephritis, chronic peritonitis, and splenomegaly with fibrosis.

The spleen measured 25 × 15 × 8 cm. and weighed 8 lbs., 4 oz. The surface was very irregular, with numerous deep depressions to which omentum was adherent. At the upper portion, a mass of splenic tissue, the size of an orange, had been almost severed from the remainder of the spleen by a deep fissure. The capsule was much thickened and covered by numerous hard irregular white plaques. The pulp was firm and brownish red. The trabeculæ were much thickened, the Malpighian bodies were not visible. The organ contained numerous fresh, and healed, infarcts. The splenic vessels in the pedicle are much thickened and calcareous.

Microscopically there was extreme fibrosis of the spleen, which contained very little of the cellular pulp. The fibrous tissue had in part undergone hyaline degeneration. The splenic veins were normal in size. The Malpighian bodies were small and showed fibrosis; the central arteries were

thickened and frequently hyaline. The infarcts showed deposits of lime and bone. The splenic veins in pedicle showed profound endophlebitis and calcification. Sections stained by Levaditi showed no treponema pallidum.

The liver was swollen and there was some fatty degeneration but no cirrhosis.

Case III. This was a case of splenomegaly associated with polycythemia in a man of 52 who had suffered from angina pectoris. For one year he had had alternating constipation and diarrhea, occasionally with blood in stools. He had lost weight and strength.

Physical examination showed the heart enlarged, the liver slightly enlarged and an enormous hard spleen reaching to the umbilicus. There was no ascites. The blood count was as follows: Red blood cells, 6,700,000; hemoglobin, 100 per cent.; leucocytes, 15,000; polymorphonuclears, 80 per cent.; lymphocytes, 20 per cent. There was no fever. A splenectomy was performed, and the patient died the following day.

The anatomical diagnosis made at autopsy was: Fresh lobular pneumonia; hypertrophy and dilatation of left ventricle; dilatation of right ventricle; chronic congestion of liver and kidneys; fat necrosis of pancreas; and recent thrombosis of the splenic veins.

The spleen measured $20 \times 11 \times 9.5$ cm. and weighed 1,190 grams. The surface was smooth except in the upper portion where there were adhesions. The organ was hard and reddish brown, and the trabeculæ were increased in size and number. Scattered throughout were numerous stellate hemorrhagic areas, conforming in outline to some of the large trabeculæ.

On microscopic examination the spleen showed a thickened capsule and marked fibrosis, the pulp being almost entirely replaced by connective tissue. The pulp cells were few in number; the splenic sinuses moderately dilated. The Malpighian bodies were reduced in size and in number. There was considerable extravasation of blood in and around some of the trabeculæ. The blood vessels in the trabeculæ showed slight thickening. In the splenic veins of pedicle there was slight endophlebitis, and in some a recent, but pre-operative, thrombosis. Sections stained by Levaditi showed no treponema pallidum. The liver showed chronic congestion with no cirrhosis.

These cases when grouped clinically fell into two classes: Cases I and II, Banti's disease; and Case III, splenomegaly associated with polycythemia.

The pathological findings common to all are the following: (1) Splenomegaly. (2) Diffuse fibrosis of the spleen. (3) A thickened and partly adherent capsule. (4) A notable diminution in size and number of the Malpighian follicles—a change in many instances due to fibrosis proceeding from the central artery. (5) An endophlebitis of the splenic veins. (6) A liver free from cirrhosis.

The characteristic clinical features of Banti's disease are the following: (1) Absence of known etiology; (2) a period of splenomegaly and anemia; (3) a transitional period in which swelling of the liver is prominent; (4) a final period in which the liver is small and there is ascites; and (5) a fatal termination by hemorrhage or anemia.

Pathologically, Banti claims the following findings as characteristic of his disease: (1) A marked fibrosis of the reticulum. (2) Fibrosis of Malpighian follicles, extending from the artery, which often shows hyaline degeneration. These changes he terms "fibroadenie," because the glandular structure of the organ is preserved. The fibrosis is not of inflammatory origin, he holds, because no fibroblasts are found in such spleens—in other words, it is a primary sclerosis. (3) A cirrhosis of the liver differing in no way from the Laennec type. (4) In many cases an endophlebitis and calcification of the splenic veins. (5) Freedom from general glandular enlargement. Banti explains the pathogenesis on the theory that the splenomegaly has an infectious origin, that the enlargement of the spleen produces a toxin which in turn causes the cirrhosis of the liver and the changes in the splenic veins, and that the anemia is due to the toxemia and to the hemorrhages.

Reviewing the clinical and pathological features of Banti's disease, it is found that there is no single feature or group of features that are pathognomonic. The criteria propounded by Banti himself do not hold on strict analysis. For instance, perfectly characteristic cases of Banti's disease have been reported for which a definite cause such as syphilis or malaria has been determined. The various stages which Banti describes also have caused frequent confusion. There have been many cases of Banti's disease (including the two reported) which showed no cirrhosis, although clinically they were in the third stage of the disease. The pathological characteristics described by Banti have been found in other conditions. Indeed practically all authorities agree that a diagnosis of Banti's disease cannot be made on a basis of anatomical findings.

Banti's theory of the origin of the disease is also unsatisfactory. It is inconceivable that a toxin should cause a splenomegaly which again causes a toxin which produces a cirrhosis in another organ. Moreover there is no reason to assume that the fibrosis is not of an inflammatory origin.

Banti believed he found confirmation of his hypothesis that the other changes were secondary to the splenic lesion in the supposed cure of the disease by splenectomy. However from a study of so-called recoveries one is hardly justified in regarding splenectomy as a cure for Banti's disease. In some cases results have been reported too soon, in others there is doubt as to whether the disease was actually that described by Banti.

A study of these three cases and of the published reports on this disease leads to the following conclusions:

1. The complex known as Banti's disease is entirely a nosological entity. It has no well-defined characteristics, either clinical or pathological.

2. Banti's disease is identical with the "splenic anemia" of Griessinger.

3. The value of splenectomy as a cure for Banti's disease must be open to question.

Discussion:

Dr. Wood: I remember one case operated on at the German Hospital—Dr. Willy Meyer would remember it. Six months later the patient showed a greatly improved condition and went back to work. This was a typical Banti's case with a very large spleen. There was a high degree of anemia which was evidently secondary, and the improvement was very interesting. At the time of operation there were two and one half million red cells and two months later the blood count was normal. Sections of that spleen are on record at the hospital. Many of these cases are very puzzling. In one recent case of splenic anemia, there was found a large gumma of the spleen. In another case of polycythemia there was a large tumor found. This was a fibrous spleen rather than one of type known as Banti's disease.

LEPROSY

W. G. MACCALLUM, M.D.

A number of lantern slides were exhibited illustrating various forms of leprosy seen in the Fiji Islands, Java, Bali, Singapore and Kuala Lumpur. The well-known histological characters of the lesions of the skin, the nerves, and the internal organs were discussed, and to some extent illustrated by the material derived from two cases which were studied at autopsy in Singapore.

Especial stress was laid upon the fact, which seems not to be mentioned in the literature, that the large vacuolated cells which form so conspicuous a feature of the lesions everywhere, and with which the bacteria are associated in such great numbers, are really cells loaded with fat globules. When stained with Sudan, the masses of tissue which form the bulk of the nodular thickenings of the skin are found to take a bright red stain, since all the cells, from the small mononuclear wandering cells through all stages to the very large foamy structures, contain in their vacuoles abundant globules of fat. Attempts to show that this fat is doubly refractive were unsuccessful with frozen sections of the formalin material, but it is possible that in the fresh state the globules may prove to be anisotropic. Further work is being undertaken to determine their exact nature. It seems possible that the fat may have some protective relation to the bacilli so that they can be reached best by fat soluble drugs.

Discussion:

Dr. Moschcowitz: I would like to ask Dr. MacCallum if he knows how frequently those who care for the lepers develop leprosy.

Dr. Ewing: I should like to ask what proportion of people in these islands have mild or unrecognized leprosy. Are there any data? Ever since the famous Early case, which was undoubtedly leprosy, I have felt that diagnosis in the early stages was difficult. I often wonder whether cases do not slip through our hands. If the early stages are not recognized it must be prevalent throughout the whole population. I wonder to what extent these cases are complicated with syphilis. Looking at these pictures I am reminded very forcibly of syphilitic lesions. I should like to know also to what extent tuberculosis is present among lepers.

Dr. MacCallum: In regard to Dr. Moschcowitz's question about the attendants, I did not wait long enough to see; but in a summary of the literature published in the first volume of the *Journal of Tropical Medicine,* very few instances are reported of people in contact with lepers contracting the disease. It appears in most cases that the contact is very intimate before infection takes place. You can't believe all the tales you hear in the East. You do hear of cases where slight contact was supposed to have caused infection, but you hear of attendants working a long time with lepers, and escaping scot free. Very few doctors or sisters of charity have been infected. One is struck at once by the apparent daring of the attendants. In the Singapore hospital they take no precautions whatever. One had been there seven years, and one nine years. At autopsy the attendants did handle body with cloths, but that was because of the disagreeable nature of the ulceration.

In answer to Dr. Ewing, I should suppose there were many mild cases but it was quite impossible for me to find that out. It would take long residence and intimate study in those countries. I saw many slight cases, recognizable only when one looked for them. The surest way of studying early cases is by studying the nasal secretions. The early lesions may be in the nasal septum. As to combination or confusion with syphilis, that seems plausible; but I think that the diagnosis of these cases in the pictures has been made on the finding of lepra bacilli. In Penang there is a large leper colony and the cases are diagnosed by bacterial study. They may have syphilis or tuberculosis as well. I had one autopsy where the patient was a leper and also tuberculous. In the Fiji Islands there is no syphilis, except in the indentured laborers who are Hindoos. In the Malay states this is not true. The syphilis observed there is beyond anything we have ever dreamed of. My first experience of leprosy was a case that had been diagnosed as syphilis by a distinguished dermatologist. She was supposed to have tuberculosis of the lungs but I am not sure it was tuberculosis. It was a very distinct leprous case.

TUMOR PRODUCTION IN ANIMALS

DUDLEY H. MORRIS, M.D.

(From Columbia University, George Crocker Special Research Fund, F. C. Wood, Director)

In spite of the wide divergence of current views on the etiology of cancer, one factor is universally admitted to be necessary in order that a transplanted tumor may grow in a mammalian host. The living cancer cell has come to be regarded as this essential unit in dealing with transplantable tumors. But that a

living cancer cell is not the primary cause but merely the vehicle of cancer becomes evident when we consider the origin of the cancer cell itself. Practically all authorities are in accord, that the cancer cell develops from some ancestral normal body cell of homologous type. And this transition from the normal to the cancerous may take place through the influence of some agent, the nature of which is unknown, acting in such a manner as to produce a permanent alteration in the normal tissues acted upon. What then is this agent, and by what means can it be studied? Obviously the best way to investigate the properties of such an agent would be to isolate it, if possible, and obtain it free from the soil where it has lodged and by its action produced the cancerous change. While this isolation of a tumor-producing agent appears to have been accomplished in a few instances in chicken tumors, it has never been done with other cancers. Rous was able by means of filtration through Berkefeld filters impermeable to ordinary bacteria, to isolate a cell-free agent capable of transmitting a chicken sarcoma from one animal to another. Repeated attempts have failed to demonstrate that the filtrates of any existing mammalian tumor can cause such growth in another animal. It is not difficult to imagine why, unless the exact conditions favorable to growth were provided, these experiments should fail, since such variants as dosage, degree of dilution, length of observation, character of tumor, etc., might well have a profound effect on the outcome.

With these possibilities in mind, experiments were carried out on a large series of animals and a number of variations in method have been employed.

In these experiments eight sarcomata, six carcinomata, and one basal-cell epithelioma were used. In nearly all cases a small amount of kieselguhr (silicious or diatomaceous earth) was injected with the filtrate, in order to furnish what Rous had shown to be the best conditions for tumor growth in the chicken sarcoma he studied.

The series comprised in all about three thousand animals, approximately two thousand mice and a thousand rats being used.

About one half the mice and one third the rats survived for five months, and many of them had nodules at the site of inoculation at this time, but no tumors had developed. These nodules when removed showed the histology of rather cellular granulomata obviously developing about the kieselguhr which was injected with the filtrate. After eight months, about one third the mice and about seventy-five rats remained alive. All the animals were negative to tumor growth except four rats in one box. These were part of a series of fifty animals which had been injected with a filtrate of the Flexner rat carcinoma to which a very small amount of kieselguhr had been added. Of this series, six animals out of fifty remained alive, four in the box in question and two in another box. The six animals, including the four animals in the positive box, had had the following treatment. They were first splenectomized, and ten days later 1 c.c. of a Berkefeld filtrate of Flexner rat carcinoma (7 gm. tumor to 70 c.c. Ringer's solution) was injected into and beneath the skin of the left flank just below the costal margin. This site was chosen merely because it was the area which had been shaved for the preceding splenectomy. To the entire filtrate (60 c.c.) were added a few drops of a milky suspension of kieselguhr in Ringer's solution. All were young animals not over four months old when injected with the filtrate. After six months these animals had been negative, except for two small opaque white nodules at the site of injection of the filtrate. These nodules were common enough in the other animals and represented merely a reactive proliferation of the subcutaneous tissue to the filtrate-kieselguhr irritant. After eight months, however, one of these lumps, still white and opaque, had reached the size of 1×1 cm. and the other, though smaller, had taken on a lobulated appearance and distinctly bluish color. In the third rat, a nodule the size of a pinhead was present at the inoculation site. The fourth animal was negative. These animals henceforth were observed daily and the following events were seen to occur. The bluish nodule grew fairly rapidly and soon surpassed the opaque fibrous one in size. It was then removed, except for a small fragment, and the greater part was

used for inoculation into seventy-two young rats, a slice being saved for microscopical examination. This tumor (Filtrate F. R. C. No. 1 [Fig. 1]), for such it proved to be, was an adeno-

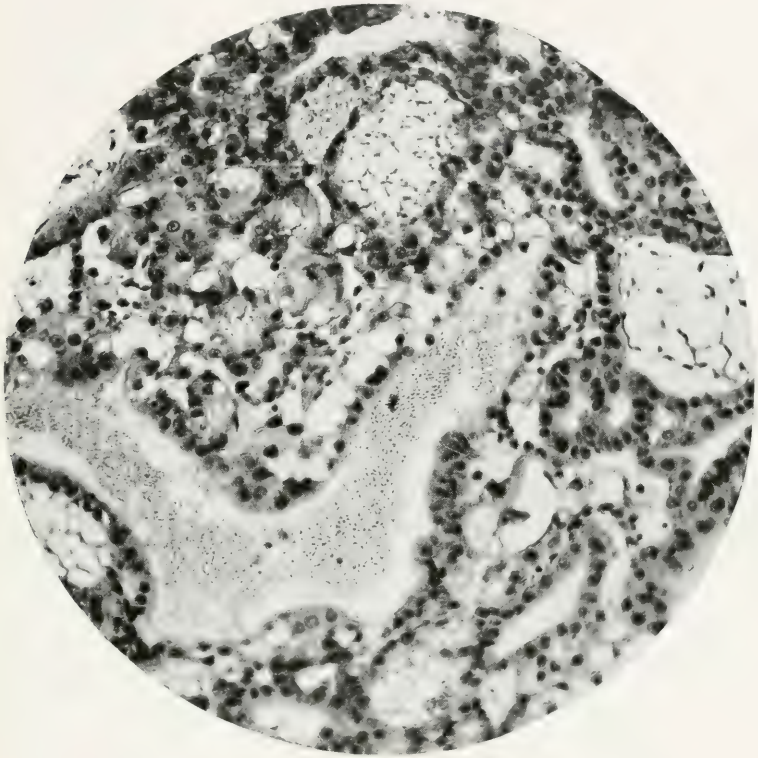

FIG. 1. Filtrate F. R. C. No. 1. (C. F. 10674). Typical adenomatous area, showing hemorrhagic character, vacuolization of cells, and production of mucus and colloid. × 200.

carcinoma of a remarkable type, totally different from the Flexner rat carcinoma and, in fact, from any rat tumor in the laboratory.

The microscopical sections of this tumor showed it to be a hemorrhagic cystadenocarcinoma with intracystic papillary outgrowths of cells and masses of cells apparently free in spaces engorged with blood. In many places the acini were very large

and their lumina empty; in others they were filled with colloid or mucinous contents. The tumor cells were relatively large, in places pyramidal or polygonal with large vesicular nuclei and resembled the cells of a serous gland. In other areas the acini were lined with a single layer of cuboidal or columnar epithelium. Here and there were scattered cells which greatly resembled the goblet cells, giving the acini somewhat the appearance of intestinal crypts. The protoplasm of the tumor cells was characteristically vacuolated and produced large and small globules of what appeared to be a colloid or mucinous substance. Certain of the cells were so distended with this secretion that they were swollen and the nucleus flattened to a mere granule at the periphery. Such cells as this, together with large aggregations of the mucinous substance, gave the tumor a characteristically reticulated appearance. In places the adenomatous arrangement was lacking and the cells showed an invasive tendency. Irregular strands or groups of polygonal cells were here found infiltrating connective tissue somewhat in the manner of scirrhous carcinoma.

During the month which elapsed after the removal of this tumor, the opaque white nodule in the second rat (Filtrate F. R. C. No. 2 [Figs. 2 and 3]) very slowly and almost imperceptibly increased in size; its margins assumed a slightly bluish tinge, and two minute bluish subsidiary nodules appeared at the lower pole. The pinhead nodule observed in the third rat (Filtrate F. R. C. No. 3), however, increased rapidly until it was larger than that in the first. It was manifestly bluish and hemorrhagic, but not much lobulated. In the meantime, a tiny nodule had appeared in the fourth rat. This, while no bigger than a grain of barley, was obviously bluish; it enlarged very slowly.

Five weeks after the removal of the first tumor, the second was removed for transplantation. Histological examination showed it to be a hemorrhagic cystadenocarcinoma of a type somewhat resembling the first tumor, but not at all like the Flexner rat carcinoma. Scattered through the tumor were foci of hyaline connective tissue in which were fibrin clot and remains of the kieselguhr which had been injected with the filtrate. Care-

ful search of serial sections failed to reveal any traces of normal breast tissue or ducts. This tumor differed from the first chiefly in the tendency for the acini to be composed of epithelium in many layers and in the more solid character of the cancerous growth.

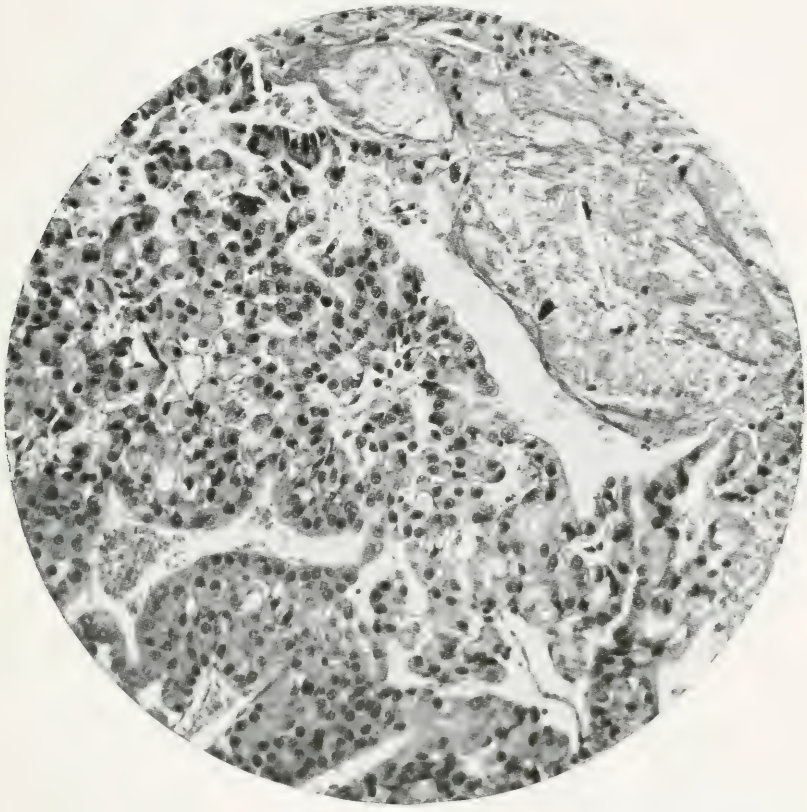

FIG. 2. Filtrate F. R. C. No. 2. (C. F. 10934.) Adenomatous area adjacent to kieselguhr remains, showing hemorrhagic character, vacuolization of cells, etc. × 200.

A few days after, the tumor of the third rat (Filtrate F. R. C. No. 3) was removed and transplanted. The sections of this showed it to be likewise a hemorrhagic adenocarcinoma, but of a type somewhat different from those previously removed. The

acini were of two kinds, some similar to those previously described, others composed of masses of stratified columnar epithelium closely investing blood capillaries.

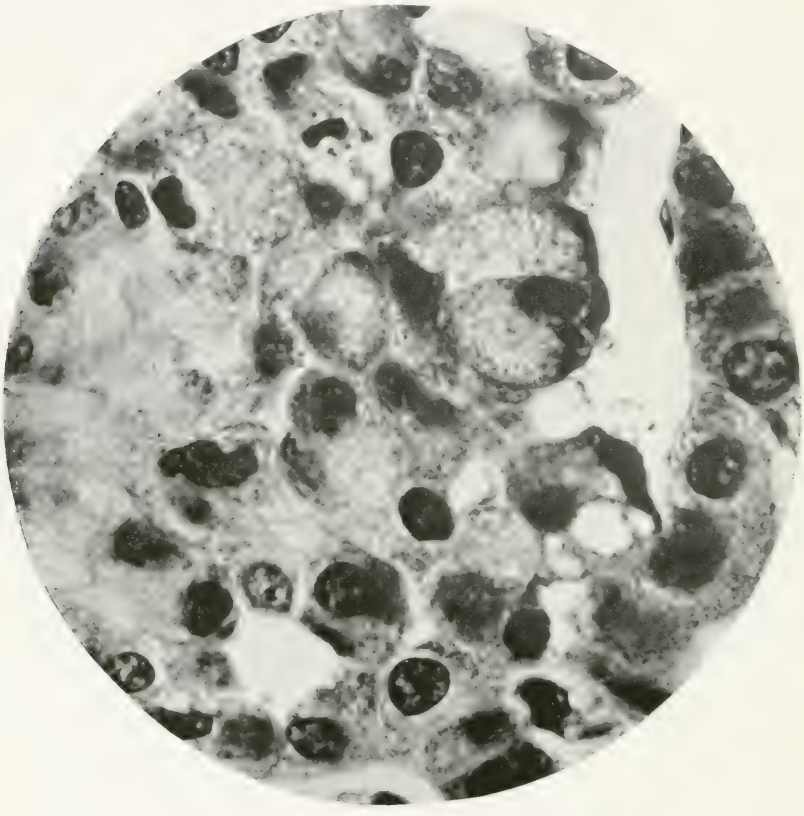

FIG. 3. Filtrate F. R. C. No. 2. (C. F. 10934.) Showing general cell characteristics, including vacuolization of cells and production of mucus. × 1,000.

The nodule in the fourth and last rat (Filtrate F. R. C. No. 4), while still very small, was removed 294 days after the injection of the filtrate. It measured roughly 4 × 5 mm., and was flattened and bluish. Serial sections showed it to be a hemorrhagic cystadenocarcinoma, resembling the first tumor. The cells were frequently vacuolated and there was abundant production

of a mucin-like substance, which in places filled the lumina of the acini.

The transplants from the first rat tumor (Filtrate F. R. C. No. 1) became definitely palpable at the end of a month and grew

FIG. 4. Filtrate F. R. C. No. 1/A. (C. F. 10978.) Transplant of Filtrate F. R. C. No. 1. Showing preservation of hemorrhagic character, adenomatous arrangement, shape, size, staining characteristics, production of mucus and colloid. × 200.

very slowly in ten per cent. of the animals. At the end of nine weeks, the largest tumor had reached the size of about 8 mm. in diameter, when it was removed for transplantation. Microscopically it showed the characteristics of the cystadenomatous part of the original tumor (Fig. 4). All the tumors of this gen-

eration preserved the hemorrhagic, nodular, and cystic character of the original tumor. It is yet too soon to observe the fate of the transplants from tumors No. 2, 3, and 4. The animal in which the second tumor arose has recently died. Autopsy showed pneumonic consolidation, but the remaining viscera showed nothing of pathological interest. There were no signs of metastases or other neoplastic growth. The spleen was absent.

There are six possible explanations of the results above recorded.

I. That through some mistake the animals had been inoculated with a transplantable tumor, either prior to or subsequent to the injection of the filtrate. This possibility may be eliminated for the following reasons:

1. The greatest care was taken in the preservation of the filtrate and use of the syringe to avoid contamination with the unfiltered tumor emulsion.

2. Each of the animals was indubitably marked by the removal of the spleen with the resulting scar.

3. The site of injection of the filtrate was the left flank, whereas the customary site for inoculation in the Crocker laboratory is the right axilla.

4. The morphology of the tumors differs from that of any rat tumors now being transplanted in the laboratory, and their hemorrhagic character is a feature rarely found in tumors that have been the subject of repeated transplantation.

II. That Flexner rat carcinoma cells have passed through the Berkefeld filter due to some imperfection in the mantle used. Against this possibility are the following points.

1. The filter was tested, before using with cultures of *B. prodigiosus* and, after using, with *B. megatherium*, and found to be impervious.

2. Red blood cells and leucocytes, both smaller than Flexner rat carcinoma cells, were found not to pass through the filter when this was tested with citrated blood. Erythrocytes, due to their flexibility in the fresh state, should be capable of passing through a very small hole should such exist. However, prolonged and

careful examination of the centrifugalized specimen failed to reveal any corpuscles whatsoever.

3. Filtrates of other tumors made with the same filters, both before and after Flexner rat carcinoma was used, failed in three thousand animals to produce tumors, which probably would have occurred had cells passed through. Many of these tumors had cells smaller than Flexner rat carcinoma cells, and some had a rate of increase and a growth adaptability greater than that of Flexner rat carcinoma.

4. The type of all the tumors found is totally different from that of the Flexner rat carcinoma. This tumor has been continuously observed in this laboratory for the past three years and so far as is known has not during that time presented an appearance comparable to the tumors found. Furthermore, the plates of the original tumor, as reported by Flexner,[1] fail to show any similarity to the present growths. It must then be assumed, on the basis of cells getting through, that, due to the exceedingly small dosage, or growth in kieselguhr granulation tissue, the tumor has changed its type from the definite regularity of the transplanted tumor to the bizarre irregularity and hemorrhagic character of the four tumors found. Furthermore, the cells must have changed in shape, size, and staining quality as well as in arrangement and must have acquired the secretion of mucus and colloid as a regular feature of their growth.

Lastly, the low percentage of takes and sluggish rate of growth of the transplants are not in the least like the Flexner rat carcinoma, which has had years of cultivation and can be depended upon to grow in nearly 100 per cent. of the stock rats, temporarily at least.

III. A third possibility to be considered is that four spontaneous tumors occurred in rats in the same box, and by chance at the site of inoculation of the filtrate. The frequency of breast carcinomata in white rats is not accurately known. McCoy's[2] statistics of 100,000 wild rats, in which complete autopsies were done, showed only 3 carcinomata of the subcutaneous tissue in the whole number. Bullock and Rohdenburg,[3] in a paper which has

just gone to press, have found 2 carcinomata of the breast in 4,300 white rats. Woolley and Wherry[4] in 23,000 wild rats found not a single instance of carcinoma.

When we consider that here are four carcinomata all arising in animals in the same box and exactly at the site of inoculation of the filtrate, it will be seen how extremely improbable it is that these could have occurred fortuitously.

IV. A fourth possibility which must be considered is that due to irritation of the kieselguhr, and independent of the tumor filtrate, portions of breast tissue have been stirred to malignant growth.

As against this possibility it may be said that:

1. Kieselguhr as an irritant has been tried by numerous investigators in the attempt to cause malignant change, but without result. It may be urged, however, that sufficient time was not given for the transition to take place. Such may be the case, but I have tried kieselguhr and salt solution alone in about 500 of the 3,000 animals, both mice and rats, on the opposite side from which the kieselguhr and filtrate was injected. One hundred and fifty of these 500 animals survived for eight months and were completely negative to tumor growth; only in the four animals splenectomized and injected with the filtrate of Flexner rat carcinoma did tumors develop. At the time these tumors were found, six animals were the only ones alive that had been treated with Flexner rat carcinoma; of these, four developed tumors. Apparently the method would bear a causal relationship to the findings.

V. The fifth possibility, that splenectomy, by removing the resistance of the animal to spontaneous tumor growth, caused the tumors, is improbable, for other rats splenectomized and treated with kieselguhr and the filtrate of several other tumors failed to develop cancer, though they survived for eight months.

VI. The remaining possibility is that we are dealing with a filterable virus capable of stimulating cells of a new host to cancerous growth.

1. Against this it may be urged that many have tried with

uniformly negative results to find evidence of such a virus in mammals. It is hardly probable, however, that the exact conditions which were found to be successful in this case have been previously fulfilled, and, indeed, the time element alone would rule out most similar experiments.

2. The sarcomatous transformation of host connective tissue[5] about certain carcinoma grafts in mice is suggestive in this connection. This intimates that there is something in the cancer nodule which is contributed by the carcinoma cells and able to influence the cells of the host, causing them to become sarcomatous. Nevertheless, it must be recalled that sarcoma development occurs after about fifty-five days in the mouse, hence it is pertinent to inquire why six months was necessary to produce tumors in rats. It is possible that this discrepancy may represent simply a difference in concentration or individuality of the agents just as one tumor differs in its rate of growth from another. Moreover, it may be that epithelial cells require a longer time than connective tissue to be stimulated to malignant growth. Finally, this power is sometimes lost, though the carcinoma continues to proliferate.

3. We know of the existence of at least one such virus in a growth reported by Rous[6] as a malignant chicken sarcoma. While this is not epithelial in character, it is probably in the realm of malignant tissue transformation and this transformation is caused by a filterable virus.

In order to clear up and extend the results here recorded, further experiments are being carried out on a large scale, and these will be reported subsequently.

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Discussion:

Dr. Ewing: I am very much impressed by the industry and care with which this work has been done. It is a distinct contribution. I am not able to agree with the statement that tumors in general are due to the action on normal cells of an external agent. I believe in the theory of cell autonomy. I think the doctor is assuming what he is trying to prove when he states that the source of the tumor process is external irritation. If, however, one starts out with this view he will feel inclined to interpret these results as Dr. Morris has done, as evidence of the artificial production of a tumor by a filterable agent. I think one would want to be careful about committing oneself to such a view. One should consider all possible sources of error as Dr. Morris has done in detail. In running over the possibilities which he mentions I would hesitate with one or two points and consider them worth further attention than he has given them. It is a well-known fact that workers who have injected various materials into various animals have encountered remarkable results, some of which have been interpreted as accidental and some in accordance with the opinion which the worker had in mind. I recall Lack's tumor from scrapings of ovarian epithelium in rabbits, and Schmidt's injections of mucor into guinea pigs. They got genuine tumors in their animals. Anyone who reads Schmidt's work must accept his conclusions if they accept his facts. Lubarsch injected embryonic cells into a guinea pig and the animal developed a remarkable tumor of the kidney. He regarded this as accidental. I don't think it is possible to set aside the possibility of pure accident in this series. It appears to me somewhat peculiar that this agent produced a different tumor from the Jobling strain. In regard to the work of Rous, a satisfactory explanation has not been given as to its true significance. I am inclined to think that if you grant all that Rous claims of a definite malignant tumor produced by a living virus, it would then be the height of folly to apply that demonstration to any other process in nature. It would be necessary to go over the whole field inch by inch and actually determine the fact if any other tumor yields the same results. It would be against all our knowledge of the wide diversity of pathological processes. All tumors are neoplastic reactions, but they are separated by hosts of factors relating to their special etiology. However, I am very pleased to have heard this contribution and I hope the doctor will be able to repeat his experiment.

Dr. MacCallum: This paper is very interesting and it seems to me that if it is true that it is one of the most important things that has been done. I am inclined to agree with Dr. Ewing that it is necessary to do more work on this line before we can speak definitely. The injection of filtrate is an important thing and I am more settled in my mind in regard to the specificity of the tumors than Dr. Ewing says he is. It is an important point that the extract should have been injected and watched for eight months and then

produced a different kind of tumor. That arouses more doubt in my mind than anything else. That very idea suggests another one, that all these tumors were in the same cage, and no animals in the other cages had tumors. Borrel observed frequently that all the animals in certain stages developed tumors and supposed them due to acarian parasites. Ehrlich ridiculed that idea, but some factor produced tumors in the one box and the others escaped. I don't believe Borrel carried out an exhaustive study on the subject, but in Paris some workers got whole colonies of animals suffering from this epidemic, as you might call it. I don't wish to throw cold water on this effort, I think it is only by such studies that we arrive at real knowledge. I hope Dr. Morris will be able to do this work over again and prove it is so.

Dr. Levin: So far as an explanation is concerned—I don't teach pathology, and I don't care about infective agents, specificity, or any other fixed idea—but I say that when we can produce a tumor the explanation can come afterwards. There is only one thing; I rise to make a motion that we demand another report of Dr. Morris whether or not this idea is proved. Borrel showed me his experiments in producing tumors in certain mice, but I heard nothing more about it. The greatest trouble we have with cancer research is that we never get to the second chapter, and that is the most important one. Success, it seems to me, always comes with the most peculiar tumor, thus we have the Rous tumor and the Flexner which was a sarcoma-carcinoma. This experiment is with 1,000 rats and the experiments should be continued on an even larger scale. Personally I would like to throw out the kieselguhr and I would not touch the spleen, but I do congratulate Dr. Morris on his results that he has obtained so far.

Dr. Wood: The spleen may not play a part at all, but it is a disturbing factor in the experiment. The logical thing to do is to repeat the experiments with and without splenectomies, and with and without the addition of kieselguhr. I think that in these cases the extract had nothing to do with the tumor production, but that a chronic inflammatory condition of the breast tissue was set up in the animals, such as occurs in chronic cystic mastitis or cystadenoma in human beings. Those tumors very closely resemble those conditions, and their morphology, in my opinion, indicates that they are primary growths. The Flexner-Jobling tumor used was derived from the seminal vesicles; and the tumors shown resemble those of the breast. It seems more probable that they arose spontaneously as a result of the irritation of the silicious earth injected rather than that the extract had anything to do with their appearance.

Dr. Hopkins: If this thing is a filterable virus, is it not conceivable that, if you transplant it into cells other than those in which it has been growing, it might produce a tumor of a new type, dependent on that of the cells in which it lodged? Ordinarily it is transplanted in tumor cells which are grafted on the inoculated animal. It is the grafted cells which multiply and consequently the new tumor is of the same type as the original.

Dr. Wood: If there is a virus, it would naturally stimulate local cells, not necessarily of the same type as the cells of the original tumor, so that it is more probable that a new tumor would be formed than that one of the original type would be reproduced. The Flexner-Jobling tumor is a well-

adapted growth of a morphology quite different from that of the tumors shown by Dr. Morris.

Dr. Morris: I realize that it is in the nature of an attack on established opinion, to produce anything of this sort. I have every intention of carrying out this work on a large scale and sifting it to the bottom.

CONGENITAL ATRESIA OF THE ESOPHAGUS AND EXTENSIVE CONGENITAL BILATERAL HYDROCELE OF THE NECK

G. L. ROHDENBURG, M.D.

(*From the Pathological Laboratory of Lincoln Hospital*)

The two specimens of congenital malformation presented here are of well-recognized types, though neither is of frequent occurrence. The history of the case of atresia of the esophagus is as follows: A healthy mother was delivered, after an uneventful accouchement, of a seven-pound female infant. From the first nursing it was noted that vomiting of unaltered milk occurred immediately after feeding. On the fifth day of life a diagnosis of esophageal obstruction was made, and a gastrostomy performed. The infant survived the operation for seven days.

At autopsy the only pathological change of interest was found in the esophagus. From the pharynx downward, there was a fusiform dilatation which ended abruptly just above the bifurcation of the trachea. From the cardiac end of the stomach, the esophagus, of normal diameter and in normal position, proceeded to the lower surface of the pouch previously described. A probe passed from the cardiac orifice passed to within two or three millimeters of the pouch where its further progress was arrested. Fluid injected from the cardiac end, even when under considerable pressure, failed to pass the obstruction encountered by the probe.

The case of congenital bilateral hydrocele of the neck has the following history. The mother, a Russian aged 39 years, had seven spontaneous miscarriages, all of which occurred about the

fifth month of gestation. Eighteen months before the birth of the present monster, she gave birth to an eight months monster identical in type with the one presently to be described, with the exception that the first monster was not as large. Both mother and father give a negative Wassermann reaction. The delivery of this monster was normal, although it occurred in the eighth month of pregnancy.

There are two types of deformity in the specimen. The least interesting abnormality is that both the upper and lower limbs are webbed. The forearm is attached to the arm by a continuous band of skin extending from the middle of the arm to the wrist. This band tightly flexes the forearm on the arm. Another band runs from the outer surface of the arm to the anterior axillary line, holding the arm tightly to the chest. The legs are flexed on the thighs by a band extending from the lower gluteal fold to about the middle of the Achilles tendon.

On the dorsolateral aspect of both sides of the neck, are two globular cystic masses which measured in the fresh condition approximately $5.5 \times 4 \times 3$ inches. These masses extend from the middle of the scapula to the occipital protuberance, and laterally to the posterior border of the sternomastoid muscle. They are separated by a groove running along the spines of the vertebræ. The cysts contained from three to four ounces of a serous blood-stained fluid. Injection into the sacs of a solution colored with methylene blue, failed to demonstrate any connection with each other, or with the spinal axis, skull cavity, thyroid or trachea. Histologically the cysts are lined by a single layer of endothelium supported by a loose edematous connective tissue. The septum between the two cyst cavities contains a large amount of elastic tissue, and is probably the stretched ligamentum nuchæ.

Discussion:

Dr. Frank: If Dr. Rohdenburg has specific proof that the preceding monstrosity born by this mother was identical with the one shown tonight the case is of considerable importance. A large number of authorities believe that hydroceles of the neck or portions of the trunk are due to contractions of amniotic bands causing pressure upon musculature and thus producing lymph statis. It seems hardly possible that the mother would have amniotic

bands occurring at the same site twice in successive pregnancies. We should have to find some other explanation for this developmental anomaly.

Dr. Moschcowitz: I performed an autopsy on a similar case, in which the upper end of the esophagus ended in a blind pouch opposite the bifurcation of the trachea, while the lower portion communicated directly with the trachea by a larger opening. This type of anomaly is by far the most common form. At that time I was unable to find an explanation of the anomaly based on embryological grounds. It is a curious fact that the common forms of congenital stenosis or atresia are at points where two tubular structures join. For example in the aorta at the entrance of the ductus arteriosus, in the duodenum at the entrance of the common bile duct, and in the intestine at Meckel's diverticulum. The fact that congenital atresia of the esophagus occurs opposite the bifurcation of the trachea could be explained in the same way.

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Owing to the fact that the Editor of the Proceedings and some of the contributors to the present number have been called into active service in France and elsewhere, it has been found impossible to complete the reports of the discussions. In some instances, therefore, these have been omitted.

PATHOLOGICAL STUDIES IN POLIOMYELITIS

W. B. BLANTON, M.D.

The basis of this study was the material obtained from fifteen autopsies on acute cases of poliomyelitis, the work being done in the laboratory of the Presbyterian Hospital during the fall of 1916. Thirteen of the cases occurred in the recent epidemic; two were earlier. Eight of the cases were from the Willard Parker Hospital, three from the Presbyterian Hospital, and one from the Roosevelt Hospital. The remaining were outside cases. All the fifteen patients were children from seven months to ten years of age; the majority were under three years. At autopsy, the bodies were usually found well developed and well nourished.

As a routine, paraffin mounted, hematoxylin-and-eosin-stained sections were used. For special study of the nervous system we employed successfully the Noguchi and Flexner, Lenhossék, and Marchi stains, and, also, in certain instances, the Van Gieson, Giemsa, Gram, and Sudan III stains.

Brain and Cord: The gross appearance of the brain was not characteristic. The cord lesions, on the contrary, in a typical case, were usually striking. There is some uncertainty in the literature as to whether or not the spinal fluid is increased in poliomyelitis, but in all of our cases the normal quantity was found, under no apparent excess of pressure. The pia covering both the brain and cord often appeared edematous; the brain and cord were frequently quite soft; and there was almost regularly a surface injection of the vessels. On section, nothing was to be made out in the brain beyond congestion, which sometimes was marked. Section of the cord, on the contrary, often showed the horns as prominent dark gray or pink H-shaped areas, frequently speckled with bright red pin-point dots. In only one case were we able to make out a distinct softening of the gray matter, such as Harbitz and Scheel have described. This occurred in the lumbar segment on one side, and after formalin fixation was the site of a definite cavity. However, certain cut

sections which grossly exhibited no change have proved to be the scene of extensive microscopic lesions. The whole cord on section had a tendency, as though under tension, to project beyond the cut margin of the pia mater, indicating a swelling which was probably due to edema and congestion.

Membranes: No changes were noted in the dura mater. The pia covering the brain was normal except in three cases. In one, we were able to see grossly a thickening and opacity of the membranes, chiefly where they dipped into the fissures. Microscopically, this case showed a meningitis over the whole brain, including the cerebellum. The predominating cell in the exudate was the polymorphonuclear leucocyte, but there were also many mononuclears. In the second case, the meninges were markedly raised from the surface of the brain as though by edema. A fair number of large mononuclear cells and red blood corpuscles were found under the meninges.

The pia mater of the cord was regularly the seat of a diffuse and perivascular cell accumulation. It was most infiltrated where it extended into the ventral fissure of the cord, and, in general, also, over its anterior surface. The nature of some of these cells was not always certain; the prevailing cell was, as a rule, a mononuclear. Some were not to be distinguished from lymphocytes; some showed larger and less dense nuclei, which were oval or round in form. Still other cells occurred with a faint cytoplasm and large oval vesicular nuclei resembling endothelial cells. Occasional plasma cells were to be observed. In some cases polymorphonuclear leucocytes were numerous; in other cases they were rare. The oxydase reaction was useful in determining the exact amount of granular cell invasion. Finally, the vessels of the pia mater were, as a rule, extremely engorged.

Vascular Changes: These may be the chief feature of the microscopic picture; or they may share about equally with the ganglion-cell change and diffuse infiltration of the horns; rarely were they relatively inconspicuous. Usually, the vessels were seen to stand out, in a perfectly characteristic way, as bright red channels crowded with erythrocytes; about them was a dense

mantling of cells. These cells, for the most part, had single, small, darkly staining nuclei, often distorted in shape from the pressure of being closely packed together. Among them, here and there, in some instances one could see large cells with round, oval, or irregular nuclei showing little chromatin. These cells all appeared to be of the same origin. We were not impressed by the presence of many polymorphonuclear leucocytes in this location until we applied the oxydase reaction. In the majority of instances, this reaction disclosed a considerable number of the granular cells which had formerly escaped notice.

The injected vessels rarely showed any disproportionate increase of white blood corpuscles, and seldom a migrating cell in their walls; while thus giving little support to the idea that these cells had their origin through the blood stream, this observation is, of course, not conclusive. There has been considerable confusion over the more or less academic question of whether or not the perivascular infiltration is intra- or extra-adventitial. Strauss, using Van Gieson's stain, claims that in the majority of his cases the cells lie between the muscle and adventitial coats, *i. e.*, intra-adventitial. We, on the other hand, found it difficult to demonstrate the adventitia in the small vessels; but in the majority of cases the cells appeared to extend beyond the adventitial lining.

Mantling of the vessels occurred in the posterior quite as extensively as in the anterior horns. In the white matter, the vessels were as regularly affected, but the greater vascularity of the horns appeared to cause the more striking effect in them. We found no evidence of the widely accepted view that a great part of the injury to the ganglion cells is due to an anemia produced by the perivascular cell accumulation, exerting pressure and compromising the lumina of the vessels. Against this is the fact that the vessels, instead of appearing compressed, are usually distended and engorged, and that the degree of ganglion cell injury and vessel mantling does not run *pari passu*.

Ganglion Cells: The ganglion cells showed the greatest variety of change, from the slightest recognizable injury to entire

disappearance. The earliest change, as demonstrated by Strauss, was a destruction of the fine neurofibrils of the nerve cell proper. The sequence of events after this was altogether uncertain, but the nerve cells swell, lose their Nissl bodies, and stain with difficulty. Their cytoplasm sometimes appeared granular, and neuronophagia seemed to be a relatively late feature of the process. All stages of neuronophagia were to be made out, from a barely recognizable increase of neighboring wandering cells in contact with the ganglion cell, to a disintegrating cell lacunated and covered by the phagocytes. Finally, nothing remained but a mass of the scavenger cells accumulated at points which some time must have been the site of ganglion cells.

Often the early changes were manifest only in a peripheral collection of the Nissl bodies. This appeared particularly true of the cells of Clarke's column, which seemed relatively immune and rarely showed extreme injury. We agree with Strauss that vacuolization is rarely seen; but nuclei excentrically placed were more frequently observed than he has admitted. The nucleus was generally the last element of the cell to show signs of injury, and often appeared unchanged in a cell whose cytoplasm stained with the greatest difficulty or showed no Nissl bodies. Frequently, the nuclei remained with just a rim of ragged cytoplasm about them. In other instances, nothing but the naked nuclei was left; this was not always true, because in some instances one found the pale swollen ganglion cells showing only a faint nucleus or none at all. Obviously degenerated ganglion cells often exhibited no signs of neuronophagia. When the process was present, the type of cell was usually the mononuclear, though in certain instances the polymorphonuclears seemed to outnumber them. The mononuclears to be observed about such a cell were of many varieties, appearing not unlike the cells to be described under the diffuse infiltration. Their nuclei ran the gamut of shape and size from the small pyknotic types to others with irregular crooked shapes and a fine chromatin network. Occasionally, we saw in such an area a horseshoe-shape nucleus pushed well to the side of the cell, with a cytoplasm obviously stuffed with recently ingested material.

As with the other changes in the cord, different levels and different sides at the same level exhibited impressive disparities in the intensity of the ganglion-cell injury. We were struck by the number of times relatively uninjured ganglion cells were to be found in the ventral groups of the motor horns. The greatest and most constant injury was seen in cells located in the center of the horn and in the neighborhood of the commissure.

Römer has pointed out that the ganglion cells are the seat of three types of injury. He described them as entirely disappeared, as washed out and homogeneous looking, or, finally, as showing neuronophagia.

Very remarkable were the ganglion cells, in which no change was to be made out by the Lenhossék stain, found occurring side by side with other ganglion cells exhibiting extreme injury and lying in a region of advanced cellular infiltration. On the other hand, we were able to see disintegrating cells in sections which were strikingly free from infiltration. This is contrary to the view held by Wickman, that ganglion cell changes are not observed where there is no interstitial change. From these observations we are inclined to the older view, held by Charcot and Joffroy, Gombault, Dejerine, and Rissler and, more recently, by Bruining, that the ganglion cell injury is not necessarily secondary to the interstitial changes of edema, congestion, cell accumulation and vascular compression as elsewhere described; but may be the result of the direct injury of the causative organisms or their toxins. This is contrary to the most recently expressed opinion of Goldscheider, Starr, Taylor, Wickman, Strauss, and others.

Interstitial Changes: A diffuse and focal infiltration was another characteristic lesion of both the anterior and the posterior horns. The focal cell collections we have described elsewhere as accumulated at the site of deceased ganglion cells. The posterior horns were usually affected along with the anterior, but the process was, as a rule, confined to the ventral half of the horn. The type of cell making up this infiltration was, again, chiefly the mononuclear. The most striking of these cells pos-

sessed a large curiously crooked and elongated nucleus without a marked chromatin content. Some of the cells were quite large, and often the section was greatly overrun with them. Small mononuclears of the lymphocyte type were generally abundant. Ranging between these and the cells with crooked elongated nuclei, were many wandering cells presenting nuclei of various forms and strongly suggestive of intermediate stages in the transition of one cell to the other. Wickman advances this idea, holding to the opinion that the large cells are polyblasts of Maximow and represent a further stage in the development of the lymphocyte. Large vacuolated cells occurred, having a horseshoe-shape nucleus near the cell border. These cells were fairly abundant in some sections, while absent from others. Fairly numerous also were cells with large oval or round vesicular nuclei displaying a very delicate chromatin arrangement.

In some cases, polymorphonuclear leucocytes appeared to be quite numerous, but in view of the number of cells with irregular and uncertain nuclei it was often difficult to decide whether we were dealing with cells of the granular series or not. To settle this point we employed the oxydase reaction as described by Winkler, and more recently by Evans. It was fairly simple by this method to see at a glance how prominent a part the granular cells played in the reaction. In some instances, they were quite, if not very, numerous; in others, it was rare to find a cell giving this reaction. In one case of thirteen days' duration no polymorphonuclear leucocytes were found, tending to confirm Robertson's contention that these cells appear early and then disappear. However, another case of only thirty-six hours' duration showed the absence of polymorphonuclears in the cervical and thoracic sections, though a fair number were present in the lumbar segment, in spite of marked lesions at all three levels. The process in the lumbar cord in this case may have been the most recent lesion. Other cases that showed many polymorphonuclear leucocytes were of two or three days' duration; while still other cases with just as brief a period of illness showed relatively few or no polymorphonuclears.

Harbitz and Scheel have described these mononuclear cells in some detail, placing them in four categories according to the form and position of the nucleus. Opinion has been considerably divided over the origin of the diffusely infiltrating cells. By Rissler, Harbitz and Scheel, and others they have been held to be leucocytes; Goldscheider and Strauss consider them of glial origin; Robertson thinks the mitotic figures he has seen in endothelial cells indicate these as the cells of origin; Marburg holds them to be leucocytes; and Wickman is inclined to regard all of these cells as of the polyblastic series. We share with Wickman the idea that the lymphocyte is the first arrival and from it the other cells are derived. This is supported by Maximow's experiments showing that lymphocytes can assume polyblastic characteristics in certain types of reaction. Further evidence is afforded in the remarkable variety of transition forms to be made out in each section.

Hemorrhage: Hemorrhage has occurred rather infrequently in this series of cases and has never been conspicuous. Wickman, Harbitz and Scheel, Strauss, Robertson, and others have described it more regularly, the latter laying great stress on it as a factor of cause in the symptomatology and fatal results.

White Matter: In cases of well-marked vascular change, the white matter strikingly shared in the process. The vessels were congested and surrounded by collars of round-cell accumulation; in one or two instances focal infiltration was seen, but it occurred in that part of the white matter in immediate juxtaposition to the anterior horns. The diffuse changes did not occur. The very loose nature of the tissues in certain cases pointed to edema of the cord as a distinct feature of the pathology; and Wickman thinks that the absorption of this explains the rapid subsidence of the symptoms. By the Marchi method, Strauss, Harbitz and Scheel, and others were able to show with difficulty an early degeneration of the axis cylinders. By the same method we were equally unsuccessful. Interest has centered around the question as to what segment of the cord is most severely injured. Among this series of cases, the cervical cord was gen-

erally more seriously involved than the lumbar segments. In the thoracic region the lesion was sometimes quite severe, but rarely as marked as in either of the enlargements.

Basal Ganglia and Pons: In these locations there were usually changes. These were oftenest a ganglion-cell degeneration with disappearance of the Nissl bodies, paling of the cytoplasm, and an excentric nucleus. Perivascular infiltration was less regularly present in these higher levels. It was quite rare to find a diffuse scattering of wandering cells. In none of the twelve cases in which the *cerebral cortex* and *cerebellum* were studied could one make out any changes save those of congestion. The hypophysis in two cases was normal.

Posterior Root Ganglia: Harbitz and Scheel found these normal. Peabody, Draper, and Dochez, Strauss, and others found that they showed the characteristic change occurring elsewhere in the cord.

Peripheral Nerves: Redlick and Monckeberg are the only writers to describe slight degeneration in the peripheral nerves. In one of four cases in which we were able to study the peripheral nerves by the Marchi method we found definite degenerative changes with darkly staining fragmented myelin sheaths. It seemed reasonable to suppose that more careful study of peripheral nerves in all cases would have led to a larger number of positive findings.

The *heart* and vessels appeared normal in every case.

The *lungs,* in six cases out of twelve, showed a moderate degree of bronchopneumonia. Congestion was a feature in only three cases. There was an acute fibrinous pleurisy in one case, and in only one was edema marked. Other than this, the lungs showed no pathological change.

The *thymus* on gross examination always appeared normal. In only one instance was it noted as enlarged. Microscopic study showed evident regressive changes in the majority of cases which were not in keeping with the hyperplasia observed by Peabody, Draper, and Dochez. This change was particularly marked in two cases. The small thymic cells often showed signs of injury

and the large phagocytic mononuclears were sometimes vacuolated and in other instances contained nuclear debris in great quantities. Pappenheimer, in his careful study of the thymus, makes it clear that this organ is peculiarly susceptible to injury, and regressive changes set in with surprising rapidity in both acute and chronic infections.

The *lymph-nodes* were usually quite red, both on the surface and on section. There were never any palpable nodes in the axilla or groin. The mesenteric nodes in six instances were noted as slightly enlarged. Twice they were definitely larger than normal. In the remaining cases they were unchanged in size. At autopsy, these alterations never seemed important when the normally large size of the mesenteric nodes of the child was recalled. Microscopically, the picture was similar to that to be described in the splenic follicles, but less marked. Edema and congestion seemed sufficient in most cases to account for the slight enlargement of the glands.

The *tonsils* from four cases were studied. In two they were apparently normal to both microscopic and gross study; in the other two there was a moderate amount of necrotic material in the crypts with a corresponding degeneration of the epithelium. The centers of the follicles showed an apparent increase in the large mononuclear cells shown generally by the lymphoid apparatus. Harbitz and Scheel found the tonsils normal, as well as the nose and throat, in three cases examined. Peabody, Draper, and Dochez thought the tonsils were usually larger than normal. Wickman and others fail to touch on this point specifically.

The *spleen* grossly was entirely unlike what we are accustomed to see in most acute infections, in that it was never soft and usually noted as rather firm. As a rule, it was not enlarged. On section the follicles usually appeared to stand out prominently on the cut surface as white translucent dots. There were several cases, however, in which the follicles were most inconspicuous. In some of the cases the organ seemed to be extremely congested.

Microscopic study has been focused on the follicular changes, and most observers have called attention to a hyperplasia. Peabody, Draper, and Dochez described endothelial proliferation at the follicular center and in the walls of the sinuses. We have been able to make out three degrees of change in the follicle. In seven cases the follicles were strikingly small and hypoplastic. In other cases, the large phagocyting mononuclears were very prominent. These cells contained large oval or round vesicular nuclei with a loose chromatin network and showed a faint staining though abundant cytoplasm. In still other cases, there were definite hyaline changes at the center. The large nuclei of the cells described, with a variable amount of nuclear detritus, appeared embedded in what was obviously the fused cytoplasm of the disintegrating large mononuclears. Frequent evidence was to be seen of the destruction of lymphatic elements in their pyknotic nuclei, fragmentation and ingestion by phagocyting cells. In general, the spleen showed a lymphatic hypoplasia rather than a proliferation of lymphocytes. Comparison of sections of spleens from cases of poliomyelitis was made with a number of sections of spleens taken from autopsies on young children who had succumbed in other diseases from one cause or another. No characteristic differences were to be observed.

Liver: Peabody, Draper, and Dochez described at some length most striking changes in the liver, and Batten has accepted their view without mentioning whether or not he was able to see the same thing. The lesions are described as focal necroses, characterized by liver cell degeneration with lymphoid and polymorphonuclear infiltration. Such changes were noted chiefly about the central veins, and they are described as sometimes difficult to find; in other cases many of them occur in a single low power field. Other observers have been content to describe merely the changes characteristic of cloudy swelling in a parenchymatous organ. In this series no instances of focal necrosis were found. In one case only did we see even a moderate number of polymorphonuclear leucocytes about the portal spaces. But many cases of acute infection and other diseases present this

picture about the vessels of the liver. Stained by Sudan III, the liver cells regularly were found to contain quantities of fat deposited in fine vacuoles. In several cases, the liver shared with the spleen and lungs in a distinct central vein stasis. Finally, as a rule, there was a moderate parenchymatous degeneration evidencing itself in swelling and the cloudy appearance of the liver epithelium, but this was not the prominent feature as one sees it in pneumonia, sepsis, and other acute infections.

The *stomach* was normal. The *large intestine* showed no pathological alteration. The lower end of the *ileum* was the seat of the principal lesion. In general, this was not striking. However, Peyer's patches were sometimes markedly enlarged, raised and red. Occasionally, they showed no change at all. The mucous membrane was always intact over these areas. Microscopically, in the submucosa engorgement of the vessels was the rule. The follicular changes were not unlike those observed in the spleen and lymph-nodes. In a few instances, the large mononuclear phagocytes were very numerous and active. But here again it was difficult to believe that there was a hyperplasia of the lymphoid structures. The very increase of the phagocytic cells was considered indicative of injured and disintegrating lymphocytes for the removal of which they were obviously mobilized. While superficially bearing some resemblance to typhoid lesions, the injury was never so severe. Ulceration did not occur and the enlargement of Peyer's patches was rarely impressive. When compared to the central nervous system, where the organism evidently does occur, the intestinal as well as other lymphoid lesions seem insignificant and hardly bespeak the presence of many, if any, organisms growing in these localities.

The *kidneys* uniformly showed parenchymatous swelling and early degenerative changes. No cases of acute nephritis were found.

The *adrenals, thyroid,* and *pancreas* were apparently normal in all cases examined.

Organisms: Flexner and Noguchi report finding organisms

without difficulty in smears of the fresh cord; they also report similar findings in sections in a few instances. Using the same stain, we likewise studied numerous sections of the cord in an attempt to find and identify organisms. We were never convinced of finding anything that could be properly called an organism. In doubtful cases the question of whether or not we were dealing with nuclear fragments, cross sections of nerve fibers, or small precipitations of stain was never satisfactorily disposed of. Harbitz and Scheel, Wickman, and Robertson all report negative findings.

SUMMARY

The *cord and brain stem* appeared to bear the brunt of the injury. The ganglion cells probably suffer independently of other elements of the cord and are among the first structures attacked by the organism or its toxins. The cells entering into the perivascular accumulations contrasted sharply with the cells of the more diffuse infiltration, having, as a rule, small dense round nuclei. Evidence of the compression of the vessels by the mantling cells was lacking. Among the interstitial changes in the cord, the presence of many cells with single elongated, often quite crooked nuclei, produced a pathological picture peculiar to this disease. We were not able by the Marchi method to demonstrate degenerative changes in the column tracks. However, the peripheral nerves in one out of four cases showed early degeneration by the same method. Hemorrhage did not seem to be the serious factor advanced by some authors.

The *lymphoid apparatus* showed an actual diminution of lymphocytes. The gross swelling and redness of the lymph-nodes and Peyer's patches were probably the result of edema and congestion. The tonsils, in two out of four cases examined, were normal. The spleen was more characteristic on gross than on microscopic examination; it was usually firm and normal in size; and on cut section large prominent follicles were the rule. The thymus regularly showed regressive changes. There was never any evidence of the hyperplasia that has been described.

The *liver* exhibited no special pathological alteration. In no cases were we able to discover the focal necroses that have been emphasized.

The *lungs,* in half of the cases, were the scene of what was obviously a terminal bronchopneumonia.

A careful search for organisms in sections of the cord gave only doubtful results.

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STUDIES ON THE ETIOLOGY OF POLIOMYELITIS

ANNA W. WILLIAMS, M.D.

Certain claims already made, and corroborated by many workers, in regard to the nature of the virus of poliomyelitis must be considered by anyone bringing forth new theories as to the chief exciting cause of this disease.

These claims are briefly as follows: First: The virus is located chiefly in the central nervous system, then in and on the mucous membrane of the gastrointestinal tract, in the adjacent lymphnodes, and in the salivary glands. It is not found, at least not to any appreciable extent, in the spinal fluid or in the blood.

Second: The virus is filterable, passing readily through Berkefeld, less readily through Chamberland filters.

Third: Of the many animals experimented with, the monkey is the only one in which a typical infection is produced by the virus.

Fourth: The virus produces an active immunity in monkeys,

and the blood serum of immunized monkeys contains substances that neutralize the virus.

All communications that do not take these points into consideration, either accepting them as true, or showing that they are not true, need not occupy our time. Only two new theories at present fulfil the requirements: that regarding the Flexner-Noguchi organism, and that regarding the streptococcus of Rosenow.

Flexner and Noguchi (1913–1915) claim for their organism, that it is found (though with difficulty) in the central nervous system of practically every monkey dying after lethal doses of poliomyelitis virus (no definite statements have been published as to the percentage of "finds" in human cases); that it has been found less often in filtrates from the central nervous system and from washings from the gastrointestinal tract; that it is slightly filterable, passing with difficulty through the coarser filters; and, finally, that it produces typical lesions in monkeys in culture generations as high as the twentieth. They have not shown that these cultures produce immunity; their chain of evidence, therefore, is incomplete as to specificity. The organism is a very minute streptobacterium growing only under anaerobic conditions. No one, outside of their own group, has corroborated their work on this minute organism (1916).

Rosenow claims that he has obtained a particularly irregular streptococcus from the central nervous system and the tonsils of all the human and monkey cases examined by him; that he has found it slightly filterable; and that it has produced typical lesions, not only in monkeys, but in many other animals. But he has not yet shown that it produces immunity. Rosenow states further that his streptococcus is the same organism as the minute coccoid organism obtained by Noguchi, and he attempts to prove this by a morphologic demonstration of the breaking off of minute forms from larger forms. He has not shown, however, that such minute forms remain minute or that they grow anaerobically; neither has he satisfactorily explained why he obtains with his cultures so many more infections in different species of

animals than with the virus. Furthermore, others have already claimed that the paralyses produced by streptococci in those animals susceptible to the virus (monkeys and, to a less extent, rabbits) are not accompanied by lesions similar to those produced by the virus. The case for the Rosenow streptococcus, therefore, is not very strong.

In our own work, we have followed, as closely as possible, the technique employed by the Rockefeller group of workers and also that used by the Rosenow group, as well as some methods of our own.

The autopsies were made under aseptic precautions as far as possible. Aseptic conditions have been most satisfactorily carried out in monkeys. The brain and then the spinal cord were removed and placed in sterile receptacles. In some cases, following Rosenow's method, before removal of the central nervous system, a portion of the surface was seared; a sterile capillary pipette was inserted; and some of the contents, both the fluid from the ventricles and the nerve tissue from different parts of the interior of the brain and spinal cord, were removed.

The material was brought immediately to the laboratory, and various culture media were inoculated from different parts, in most of the cases, in the order given:

1. Veal agar plates with and without 0.2 per cent. glucose.
2. Extract broth plus 0.2 per cent. glucose.
3. Veal broth.
4. Slant egg medium with and without ascitic fluid.
5. Slant Bordet medium with and without ascitic fluid.
6. Veal horse serum agar tubes with and without 0.2 per cent. glucose.
7. Ascitic fluid with kidney tissue (prepared as recommended by Noguchi).

Many of the tube cultures were covered with albolene, and some were placed in an atmosphere of nitrogen as well. From twenty to two hundred initial cultures were made from each case, and were put in an incubator at about 35° C. After these were made, material was taken for filtering and some for inocu-

lation of monkeys and rabbits, some was dropped into fixatives for sections and some put into 50 per cent. glycerin and stored in the ice-box at about 40° C. for later examinations. The remaining material from many of the good autopsies not used by other workers, was sent sealed to a cold storage plant.

As the following table shows, we had material from 40 human autopsies. In one case the condition was proved not to be poliomyelitis and this served, therefore, as a control. Of the thirty-nine cases, twenty-six showed in smears a minute streptobacterium morphologically similar to that obtained by Flexner and Noguchi, twenty-three showed irregular streptococci, and eighteen showed other minute organisms which are still being studied—but all of these in only a few out of many tubes. The majority of the tubes remained sterile.

It was comparatively easy to obtain pure cultures of streptococci from most of the tubes showing them in smears, as well as pure cultures of several of the minute organisms not like the Flexner-Noguchi organism; but from the mixed cultures showing morphologically the Flexner-Noguchi type of organism it has been impossible to obtain any anaerobic organism of the Flexner-Noguchi type. Only twice so far have we obtained pure cultures of a microorganism answering to the description of their organism, and in these cases from tubes that originally contained pure growths. We, fortunately, are able to compare these organisms with those of the Rockefeller strains, since Dr. Amoss kindly gave us four strains of the Flexner-Noguchi germ and they are being closely studied now. So far, they seem similar, except that one of them is growing rather more slowly than the others. They grow very abundantly in 0.2 per cent. glucose veal agar plus horse serum. In this medium they are extremely minute.

Up to the present time we have made autopsies on fifteen monkeys inoculated with the virus, and though we have found in five monkeys morphologically typical minute organisms mixed with medium-size streptococci, we have not yet obtained pure cultures of the Flexner-Noguchi type of organism from any of these monkeys.

When we first found these minute cocci in the smears from the primary tubes mixed with other organisms, we were inclined to believe that this type of organism was present very frequently. On the other hand, since we have been carrying on pure successive cultures of streptococci under anaerobic conditions, we have found, as did Rosenow, that most strains produce some forms as minute as those of the Flexner-Noguchi type, and have come to the conclusion that we can judge very little as to whether this minute organism is present from a morphologic study alone of mixed cultures from these cases. Why we are not getting the minute organism from these monkeys, if it is invariably present, we can not understand. Our pure strains are growing abundantly in our ascitic fluids, proving that it is suitable, and we are inoculating many tubes of the material, but most of the tubes remain sterile. We have not obtained this organism from any filtrate.

In regard to the type of streptococcus found, the majority answer to the description of Rosenow; but we have found at least three distinct varieties, among them a *Streptococcus hemolyticus* from two of the monkeys.

Culture Controls: The largest numbers of our cultures were made in media to which animal tissues were added; therefore, all media had to be most carefully controlled. We found streptococci in our control tubes several times, and even more frequently found a small, irregular bacillus with many minute forms. This grew rather slowly, so that the early examinations did not show it. Occasionally, whole sets of our animal tissue cultures were contaminated by small organisms of this type.

ANIMAL EXPERIMENTS WITH CULTURES

One of our strains, resembling the Flexner-Noguchi organism, was inoculated into a monkey on the third culture generation and produced in twenty-four hours a spastic paralysis in the arm opposite the lesion. In forty-eight hours the leg on the same side as the arm became stiff. Later (two or three days), the condition in the arm improved, but the leg be-

came weak. The leg continued weak for several weeks, and is still being dragged slightly. This animal was inoculated later with a fixed monkey virus and showed no immunity.

Results from Primary Cultures of Fresh Material

	Smears			Pure Cultures		
	Minute Streptobacterium Morphologically similar to Flexner-Noguchi	Streptococcus	Other Minute Organisms	Streptobacterium	Streptococcus	Other Organisms
Human cases: 8..	+	+	+		1	4
10...	+	+	−	2	7	
4...	+	−	+			2
4...	+	−	−			
3...	−	+	+			1
2...	−	+	−			
3...	−	−	+			1
5...	−	−	−			
Monkeys: 3.....	+	+	+		2	
2.....	+	−	+			
4.....	−	+	+		2	
5.....	−	+	−		4	
1.....	−	−	+			
Controls						
Human: 1.....	−	−	−			
Monkeys: 2.....	−	+	−		1	
Dogs: 1.....	−	+	+		1	
Cats: 1.....	−	−	+			
2.....	−	−	−			
Rabbits: 5.....	−	+	−		2	
1.....	−	−	−			

In our cultural work so far we have been much hampered by the lack of monkeys. For this reason we could not test our later culture generations until they were too old to promise success. We have also been able to make only a few tests with streptococci, but, of the three monkeys inoculated with cultures obtained after the method of Rosenow, none have shown paralysis. Two rabbits out of twelve inoculated with streptococci showed paralysis with localization of the streptococci in the central nervous system.

Our results up to the present may be summarized as follows:

1. A minute organism similar to the one described by Flexner and Noguchi has been isolated from the central nervous system of a very small percentage of cases of human poliomyelitis. No similar organism has been isolated from any of fifteen monkeys dying after inoculation with poliomyelitis virus.

2. No new evidence has been obtained for or against the specificity of this organism in the etiology of poliomyelitis. The organism is a species distinct from that of any aerobic streptococcus studied.

3. Streptococci of several types, those answering to Rosenow's description predominating, have been found in small numbers in the majority of these cases.

No evidence has yet been found indicating a specific etiologic relationship of any of these types to poliomyelitis.

BACTERIOLOGICAL NOTE ON ACUTE ANTERIOR POLIOMYELITIS

CHARLES NORRIS, M.D., F. I. SHATARA, M.D., AND R. T. DEAN, M.D.

The purpose of this brief report is to record the bacteriological examinations of the spinal fluids and of the brain and spinal cords made during the recent epidemic on cases of acute poliomyelitis admitted to the wards of Bellevue Hospital. Our results have been absolutely negative and we wish, therefore, to emphasize the fact that the negative character of our examinations is in accord with those made by previous observers, with a few exceptions, until this last summer.

The reports of Rosenow and his co-workers, Nuzum and Mathers, however, may serve to justify this presentation. The detailed report will be published later.

Eighteen spinal fluids were cultivated.

Technique: Nutrient 1 per cent. peptone agar, 1 per cent. acid to phenolphthalein, with the addition of 2 per cent. of dextrose, was used. In these melted tubes, sterile pieces of rabbit

kidney and sterile ascitic fluid were added, and after inoculation with the spinal fluid, were rapidly cooled and topped with sterile paraffin of high melting point. Six of the eighteen cases were contaminated, as shown by diffuse clouding of the medium and gas formation. In ten cases, the tubes remained sterile for long periods of time. One to two c.c. of spinal fluid were introduced into each tube and as many tubes as possible were inoculated.

Our reason for not using the fluid media which has been so intensively recommended was the obvious risk of contamination, and, also, the conviction, derived from past and extensive experience, that a solid medium is as favorable to the cultivation of microorganisms as is a fluid medium. After the appearance of reports in the press of the presence of cultivatable microorganisms in the spinal fluids of cases of acute poliomyelitis, the fluid media recommended were employed and aerobic and anaerobic cultures made. Except for the presence of contaminating organisms our results were negative.

The brains of two patients dying of acute poliomyelitis were cultivated. No growth was obtained, all the media mentioned above being used, both before and after filtration. A number of animal inoculations were made, namely, intracerebral and intraspinal, with the spinal fluid and the material obtained from pieces of brain. No paralysis or deaths occurred, except from accident. We also inoculated white rats intraperitoneally with the material from autopsies and with the spinal fluids, and a few wharf rats, all with negative results.

We wish to call attention to the gradual development of opalescence in a considerable number of our ascitic-agar tubes inoculated both with the brain material and with the spinal fluids. The opalescence resembled that described by Flexner and Noguchi, and a somewhat extended study of this phenomenon was made. Briefly, opalescence did not appear in tubes which were finally determined to be sterile. In a few tubes, however, we were considerably puzzled over the late development and gradual extension of the opalescence from the lower part of the tube to a zone, say, approximately 1 c.c. from the paraffin. In these ex-

hibits a few small bacterial colonies were invariably found about the kidney fragment near the bottom of the tube. In our opinion, the opalescence is due to the fermentation of the dextrose induced by the growth of bacteria, causing an acid precipitation of the proteins of the ascitic fluid.

Studies were made of the sections of fifteen cords and brains gathered from various sources. Some of the cases diagnosed as poliomyelitis presented no evidence of the disease at autopsy or later, on section, and in these cases the condition was found to be a meningitis, a gastroenteritis, or an acute intestinal autointoxication, and, in one case, a glioma of the cerebellum.

The two cases studied bacteriologically presented typical microscopic lesions of anterior poliomyelitis. Sections from six cases of poliomyelitis were stained by Gram, Weigert, and methylene blue. In 50 per cent. of the cases a few Gram-positive cocci arranged in pairs and clumps, resembling staphylococci, were demonstrated. Six normal cords picked out at random were also cut and stained, the sections presenting no pathologic lesion. In half of these normal cords, cocci, somewhat similar to those described above in sections of the poliomyelitis cords, were demonstrated. Although we speak of finding in 50 per cent. of our poliomyelitis cases a few Gram-positive cocci in pairs and clumps, it was only by the most careful search that these were discovered; and never more than one small clump was found in any one section, usually there was only an isolated diplococcus.

EXPERIMENTAL STUDIES ON LYMPHOCYTES.

CYTOTOXINS FOR THYMUS AND TONSIL LYMPHOCYTES

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We have been engaged during the past months in studying the behavior of tonsil lymphocytes and of the small cells of the

thymus gland under a variety of experimental conditions, and wish this evening to present certain of our experiments which we hope may prove of interest from the serological viewpoint.

We wish first to outline the very simple technique which has been employed, and to illustrate its applicability to different problems.

We have used as a criterion of cell injury the diffuse staining of the cytoplasm and nucleus with trypan-blue. That this increased permeability to the dye is a delicate indicator of cell injury has been recognized by Gross, McCurdy and Evans, Evans and Schulemann, Rous and others. It seemed, therefore, an obvious procedure to apply this index in a quantitative way to a study of the resistance of lymphocytes under varying conditions. Our principal source of material has been the cells of the rat thymus, which can be conveniently obtained in a uniform suspension practically free of red blood cells and fixed reticular elements. Incidentally, we have, as we shall show later, obtained evidence of the biological identity of the small thymus cells with the lymphocytes derived from lymph-nodes. Freshly excised human tonsils have also been used to obtain suspensions, but it is more difficult to get them blood-free, and we have on the whole preferred to work with the rat thymus cells.

Suspensions of these cells in 0.85 per cent. salt or in Locke's solution are subjected to various agencies or environmental conditions, and after a given period, trypan-blue is added in a concentration varying from 1 to 10,000 to 1 to 5000. A drop of the stained suspension is then placed in a counting chamber, and the percentage of stained cells determined by counting 500 or 600 cells. One obtains in this way a quantitative measure of cell injury, comparable in its delicacy to the hemolytic reaction. By developing a routine method of procedure, paying careful attention to details, one is able to reduce to a considerable degree the factors of error inherent in the method. It is not possible, however, to set up standard figures. Suspensions made from different animals show very different percentages of stained cells under similar conditions, and it is essential in a given experiment

to use, as control, suspensions of the same animal maintained under comparable conditions.

We have with this technique studied the resistance of lymphocytes to hyper- and hypotonic solutions, to variations in H-ion concentration, to asphyxia, to variations in temperature, to photodynamic substances, such as hematoporphyrin, to various chemical agencies such as the anesthetics (ether, chloral hydrate, urethane), to potassium cyanide, sodium oleate, and saponin; and to the *x*-rays. We have found that serum and other colloids exert a distinct protective action upon the cells. The cells of starving and of diseased or emaciated animals have shown greatly diminished resistance, and the cells of young rats are distinctly less fragile than those of older animals.

The method has been found serviceable in the study of cytotoxins, and although our work is still very incomplete, we desire to present at this time the results which have thus far been obtained.

Rabbits were immunized by repeated intravenous injections of washed thymus and human tonsil lymphocytes. The sera of rabbits, when inactivated and reactivated by the addition of complement, develop marked toxicity for the lymphocytes used as antigen. This is evidenced by the great increase in the proportion of stained cells, but is equally manifest in the pronounced morphological changes which are produced. The cells become very irregular in shape, and the nuclear material may fragment into small refractile particles. Some of the cells show a different type of alteration, namely a hydropic swelling of the cytoplasm. The cell membrane becomes very distinct, and the small nucleus is surrounded by a clear watery zone.

In addition to these cytotoxins, the specificity of which we shall discuss presently, there are developed agglutinins. The lymphocytes become aggregated into clumps which adhere to the side of the tube or sink down to the bottom, leaving the fluid clear in contrast with the milky control suspension. The agglutinins are thermostabile, at least to temperatures of 58° to 60°.

The immune anti-thymus and anti-tonsil sera were tested

against rat and human erythrocytes, and found to be hemolytic and hemagglutinative in dilutions of 1 to 25. The hemolysin and hemagglutinin were completely absorbed by exposing the sera to thymus cells. On the other hand, the thymo-toxic factor and the agglutinin for thymus cells appear to persist after exposure of the serum to washed erythrocytes for eighteen hours in the ice-box. These experiments seem to indicate, therefore, that the lymphotoxic and agglutinative factors are distinct from the hemolytic and hemagglutinative ones. At any rate, the conditions of the experiment eliminate the source of error which complicate those experiments which have been designed to demonstrate the specificity of cytotoxins by injecting immune sera into animals. The lesions under such conditions may, as Pearce and others have pointed out, be secondary to the hemolytic and hemagglutinative actions of the sera. In the test tube, this source of confusion is obviated.

We have immunized a rabbit against washed rat erythrocytes and obtained a serum which is strongly hemolytic and causes partial hemolysis in dilutions up to 1 to 400. This same serum upon the addition of complement after inactivation is also agglutinative and toxic for rat thymus cells. We have, however, succeeded in completely absorbing out the hemagglutinative and hemolytic factors, and in demonstrating that the thymoagglutinin and thymotoxin persist. A rough quantitative comparison of the amount of thymo-agglutinin in the original and absorbed serum shows that it persists undiminished. This would appear to show convincingly that we are dealing with distinct substances.

It has, of course, been of interest to compare the effect produced by the immune anti-thymus serum upon thymus cells *in vitro* with the lesions produced by injecting the serum into the living animal. Our first experiment, in which we injected 1 c.c. of the native serum, after previously removing a bit of the thymus tissue for histological control, was negative. No lesions were caused in the thymus, and the percentage of stained cells in suspension to which trypan had been added, was not abnormal.

When, however, the serum was first inactivated and guinea-

pig complement simultaneously injected, the results were very clear cut. The experiment in detail was as follows:

On December 21, two well-nourished young rats of approximately the same age and weight were etherized, and a fragment of thymus was excised from each. Some of this tissue was examined histologically and found to be normal. The remainder was suspended in Locke's solution and counted in the usual way with trypan-blue.

Rat *A* (reserved as control) showed 5.1 per cent. of stained cells,
Rat *B* showed 9.0 per cent. of stained cells.

Five days later, the wounds being uninfected and the animals in good condition, Rat *A* (control) was injected intraperitoneally with 1.5 c.c. of normal inactivated rabbit serum plus 1 c.c. of guinea-pig complement (1 to 10). Rat *B* received 1.5 c.c. of anti-thymus serum plus complement. After twenty-four hours the rats were killed, and suspensions made from a portion of the thymus, the remainder being fixed and sectioned.

The counts showed the following:

Rat *A* (control) 12 per cent. of stained cells,
Rat *B* 41 per cent. of stained cells.

The histological study of the thymus from the rat injected with anti-thymus serum revealed a striking atrophy of the tissue. The cortex was rarefied; the small thymus cells were pycnotic and fragmented; and numerous partially decolorized globular masses of chromatin were found in and amongst the reticular cells. The thymus from the rat injected with normal rabbit serum showed no changes.

There is, thus, in this experiment a complete parallelism between the action of the serum *in vivo* and *in vitro*.

There is another question upon which our work, we believe, has a bearing, namely the identity of the small thymus cells with the lymphocytes of lymph-nodes and those of circulating blood. For many years, as you are aware, there has been discussion as to whether the small thymus cells are epithelial in origin or whether they are lymphocytes which at an early stage wander in

amongst the epithelial cells from the adjacent mesenchyme. The question has been threshed out on morphological grounds. Stoehr, Mietens, Schridde, Aschoff, Fulci, and others still maintain the epithelial nature and genesis of these cells as against the counter opinion led by Hammar and Maximoff. It is admitted by all except Schridde, that thymus cells are morphologically identical with lymphocytes to the last detail, and their active mobility and great sensitiveness to the *x*-rays are further characteristics in common.

We have been able to bring still further evidence of the biological identity of thymus and lymph-node lymphocytes by comparing their behavior to immune anti-thymus serum. The serum was found to be equally agglutinative and toxic against cells derived from thymus or from mediastinal lymph-nodes. It is difficult, working with rats, to obtain sufficiently dense suspensions from the small lymph-nodes for absorption experiments, although it will doubtless be possible in this way to bring still more convincing proof of the identity of the two types of cells.

If the thymotoxic action of our hemolytic serum, which we have shown to be distinct from its hemolytic activity, is attributable to the simultaneous injection of small numbers of circulating lymphocytes, this would suggest strongly that these blood lymphocytes are also akin to the thymus cells.

In conclusion I wish to express my thanks to Miss Kate Brogan for her very able assistance in the work.

A MODIFIED INTRADERMAL TEST IN RABBITS FOR DETERMINING THE VIRULENCE OF DIPHTHERIA BACILLI

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In a previous communication before the New York Pathological Society, I presented a simple and economical method for

testing the virulence of diphtheria bacilli on guinea-pigs. With this method two large guinea-pigs are used for determining the virulence of four different strains of diphtheria bacilli, one animal serving as a control and receiving 1.0 c.c. antitoxin (about 500 units per cubic centimeter) intraperitoneally at the same time. Fresh twenty-four-hour Loeffler cultures of the various strains are uniformly suspended, each in 20 to 25 c.c. of salt solution; and 0.2 c.c. of each suspension is injected *intradermally* into the depilated abdominal skin of a guinea-pig. The abdominal surface is divided for this purpose into four areas, and the injections are made as far apart as possible. The results of the tests are noted every twenty-four hours for four to five days. Virulent strains produce a definitely circumscribed local inflammatory lesion, which shows a superficial necrosis in two to three days. In the control pig, the skin at the site of the test remains normal. With non-virulent strains, no lesion will be found in either control or test animal.

Recently, while carrying on some work with toxin-antitoxin in rabbits, I used the intradermal test with diphtheria toxin to determine the development of an active antitoxic immunity in these animals. The accurate results obtained with the toxin test, and the sharply circumscribed skin lesions produced by the toxin when no antitoxin was present suggested the use of rabbits for determining the virulence of diphtheria bacilli. For this purpose the back and flanks are shaved, or the animals are anesthetized, and the hair is pulled out. Six intradermal injections can be easily made on one animal. Similar injections are made in a control animal that has received 500 to 800 units of antitoxin intravenously. The bacterial suspension is prepared in exactly the same way as for the test in guinea-pigs. If the strain is virulent a local indurated and necrosing lesion will develop at the site of injection, while the control test will show no lesion.

As a rule, if the strain is non-virulent no lesion will develop. Occasionally with non-virulent strains a temporary redness and slight infiltration appear at the site of injection in twenty-four hours, which disappear, however, in another twenty-four hours.

Repeated comparative tests made on guinea-pigs and rabbits with numerous virulent and non-virulent strains have shown an absolute analogy in the results.

The advantages of using rabbits in preference to large guinea-pigs are not many. Six strains can be easily tested on each rabbit instead of four; again, rabbits are a little more resistant to diphtheria toxin than guinea-pigs, and a somewhat larger number of bacteria can be injected in each area, without the danger of killing the animals; and, further, the areas of injection are a little further apart, thus avoiding a possible overlapping of lesions. At times, also, guinea-pigs are quite difficult to obtain, and rabbits may have to be used for this purpose.

Discussion:

DR. PAPPENHEIMER: How long does it take the lesion to develop?

DR. ZINGHER: Sometimes the lesions are quite distinct at the end of seventy-two hours. At first, there are redness and infiltration, with gradual progress to necrosis, perhaps at the end of the third or fourth day. The control guinea-pig can be used for another set of tests.

DR. NORRIS: Were these pure cultures or mixed cultures from the throat? The reason for bringing up this point is that I think everyone has found considerable difficulty in isolating diphtheria bacilli from mixed throat cultures. Streptococcus colonies look much like diphtheria colonies on a plate. In the rabbits shown, I can not see very much difference in the effects of the different cultures.

DR. ZINGHER: They were pure cultures. It is important to have Loeffler tubes with a uniform surface well covered. The rabbit was injected in six places with four virulent and two non-virulent strains; at the latter areas the skin shows no necrosis. There is some dermatitis, due to the rabbit having been kept on the roof, where it was cold. We have very little difficulty in getting pure cultures. What we do is to take the culture and inoculate a fluid ascitic broth or serum broth culture, and after twenty-four hours make a plate from the pellicle. One assistant obtained pure cultures very uniformly —about nine out of ten.

THE ADVANTAGES OF TRIKRESOL IN ANTITOXIC PLASMA AND ITS EFFECTS ON CONCENTRATION

EDWIN J. BANZHAF, M.D.

Trikresol or carbolic acid, when added to citrated or oxalated diphtheria or tetanus antitoxic plasma, prevents the solidification that frequently takes place, especially on long standing. When solidification takes place, it necessitates the breaking of the bottle and the grinding up of the mass. Even with this procedure, it is hardly feasible to recover more than half the original amount of fluid.

When trikresol 0.4 per cent. is added immediately after the plasma has been decanted or drawn off from the cells, it can be kept indefinitely without the slightest solidification taking place.

Plasma with the trikresol added allows a further separation of non-antitoxic proteins than plasma without trikresol. The concentration process is essentially the same, *i. e.*, the plasma is diluted with water, and thirty per cent. saturated ammonium sulphate solution is added. This mixture is heated rapidly up to 57° C., and then the temperature is gradually increased to 63° C., requiring about two hours. The hot mixture filters very rapidly (ten liters through a 50 cm. paper on ribbed funnel in less than two hours). The filtrate is brought up to fifty per cent. saturated ammonium sulphate solution and carried on as described in my former publications on purifying and concentrating antitoxins. The plasma trikresol combination gives a higher purified end product than is obtained by the former methods, and one which does not cloud on long standing.

COMPARATIVE TESTS OF ANTIGENS AND OF INCUBATION TEMPERATURES FOR THE WASSERMANN REACTION

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Abstract

Tests were performed by six different methods upon 501 identical specimens from 457 patients. Three antigens were employed: cholesterinized alcoholic extract of beef heart, simple alcoholic extract of beef heart, and the acetone-insoluble lipoid fraction of alcoholic extract of beef heart, prepared according to the method of Noguchi. Each of these antigens was used at two different incubation temperatures for fixation of the complement, 37° C. and 8° C., the subsequent incubation after addition of sensitized erythrocytes being carried out at the higher temperature.

With specimens from patients probably syphilitic (some of the untreated cases) the cholesterinized antigen at 8° C. gave the largest number of positive reactions, being followed in order of efficiency by the plain antigen at 8° C., cholesterinized antigen at 37° C., acetone-insoluble antigen at 8° C., the same at 37° C., and, last, the plain antigen at 37° C. The results obtained in this group of patients are summarized in Table I.

TABLE I

Summarized Results of 142 Tests on 123 Patients Probably Syphilitic

Antigen and Temperature	Positive		Doubtful		Negative	
	Number	Per Cent.	Number	Per Cent.	Number	Per Cent.
1. Cholesterinized at 37° C.	93	65.5	3	2.1	46	32.4
2. Simple extract at 37° C.	54	38.0	4	2.8	84	59.2
3. Acetone-insoluble at 37° C.	66	46.5	0	0.0	76	53.5
1. Cholesterinized at 8° C.	119	83.8	4	2.8	19	13.4
2. Simple extract at 8° C.	104	73.2	6	4.2	32	22.6
3. Acetone-insoluble at 8° C.	86	60.6	6	4.2	50	35.2

With 16 of the 501 specimens, some degree of fixation was obtained in one or more of the tests, which we have been compelled to regard as false positive reactions. The tests are enumerated in Table II. The tubes containing the antigens are numbered 1, 2, and 3 and the anti-complementary control is numbered 4. It will be noted that such false reactions were most frequently obtained with the cholesterinized antigen at 37° C.

TABLE II

Data of 17 Tests on 17 Patients, Probably Not Syphilitic, in Which Some Degree of Fixation Was Observed

No.	Clinical Data	Complement-Fixation							
		At 37° C.				At 8° C.			
		1	2	3	4	1	2	3	4
8862	M 30, Jaundice, enlarged liver	2+	—	—	—	2+	—	—	—
8868	F 22, Acute articular rheumatism	2+	—	—	—	1+	—	—	—
8869	M 40, Jaundice, ascites	—	—	—	—	4+	—	—	—
8870	F 26, Lumbar pain	—	—	—	—	1+	—	—	—
8871	M 20, Hysteria	—	—	—	—	1+	—	—	—
8887	F 6, Cretin	1+	—	—	—	—	—	—	—
8897	M 24, Typhus fever	4+	—	—	—	—	—	—	—
8898	F 37, Duodenal ulcer	3+	—	—	—	—	—	—	—
8899	F 25, Endocervicitis	2+	—	—	—	—	—	—	—
8963	F 3, Mental deficiency	1+	—	—	—	—	—	—	—
8970	F 23, Catarrhal cholangitis	4+	—	—	—	—	—	—	—
8976	F 8, Miliary tuberculosis	1+	—	—	—	—	—	—	—
9152	F 2, Idiocy, father luetic (?)	4+	—	—	—	4+	—	—	—
9338	F 25, Two babies died young	—	—	—	—	4+	—	—	—
9364	M 20, Prepatellar abscess	4+	—	—	—	—	—	—	—
9386	F 30, Cervical adenitis	—	—	—	—	4+	—	—	—
	Positive	8	0	0	0	5	0	0	0
	Doubtful	3	0	0	0	3	0	0	0
	Negative	5	16	16	16	8	16	16	16

Of the 501 specimens, 341, from patients evidently not syphilitic, gave no degree of fixation in any of the tests.[1]

[1] For a detailed report of this series of tests see Jour. Infect. Dis., 1917, xxi, 233.

ANEURYSM OF THE CORONARY ARTERY

H. S. MARTLAND, M.D.

(*From the Pathological Department of the City Hospital, Newark, N. J.*)

Aneurysm of the coronary arteries is exceedingly rare, and this fact warrants the demonstration in a brief manner of a heart presenting such a lesion.

The specimen was obtained by Dr. Warren, Coroner's physician, at a medicolegal autopsy. An apparently healthy negro woman, 32 years of age, was found lying dead on the kitchen floor of her home. The only history obtainable was that she had complained for some time of vague epigastric and precordial pains. On opening the pericardium, the cavity was found to contain a large red blood clot the size of a man's fist. Numerous old pericardial adhesions were present. The source of the pericardial hemorrhage was found to be a linear rupture, about 1.5 cm. in length, situated in a globular mass 6 cm. in diameter, which occupied the right one half of the coronary sulcus.

The heart was greatly enlarged, due mainly to hypertrophy; it weighed 480 gm. The right heart was negative except for hypertrophy. On opening the left heart the endocardium was seen to be free and clear except at the base of the aortic ring, where it was grayish in color and slightly thickened. The mitral valve measured 11 cm. in circumference and the cusps were free and clear. The aortic valve measured 7.5 cm.; its cusps were distinctly wrinkled, stiffened at their bases, and retracted. The left ventricle measured 2 cm. in thickness without trabecula, the muscle was light red in color, and firm, and near the apex showed a few grayish streaks and patches. The aorta just above the aortic valve presented a saccular, aneurysmal dilatation or pouch which measured 2.5 by 4 cm. in diameter and 1.5 cm. in depth, the right anterior aortic sinus formed its bottom. This pouch showed numerous yellowish, slightly raised, round, oval, and irregular patches, some of them showed slight and moderate calcification

(early and superimposed atherosclerosis). In addition, however, the pouch and also the surrounding aorta for a distance of 4 cm. above the aortic ring showed a rubbery, irregular, but smooth

FIG. 1. Coronary aneurysm. Posterior surface of heart showing aneurysm of the right coronary artery measuring 6 cm. in diameter. Aneurysm outlined in white. A bristle passes through the rupture.

appearance; the intima was slightly cloudy and there presented numerous white, hyaline-like, grayish and grayish-blue, slightly

raised areas, many of which showed small depressions or were wrinkled or presented distinct puckered scars (chronic productive mesaortitis of Heller and Döhle). The right or posterior coronary artery arose from the right anterior aortic sinus at the

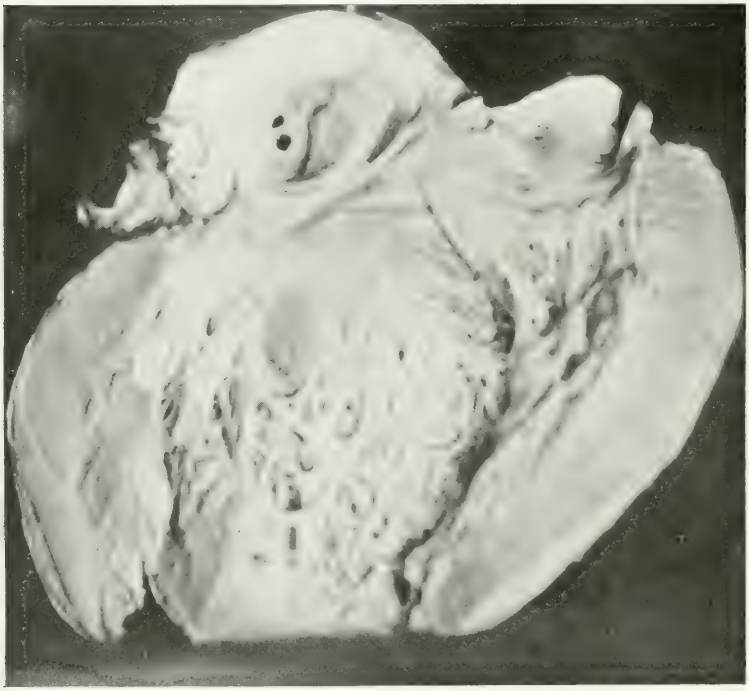


FIG. 2. Coronary aneurysm. The right aortic sinus is the seat of an aneurysmal pouch. The right coronary artery shows two openings, both of which communicate with the aneurysm behind. The aorta and the aortic cusps are involved in a luetic lesion.

bottom of the aneurysmal pouch and showed two separate openings in the aorta; each was round, one being placed above the other; they measured 4 mm. in diameter and were separated by a bridge of aortic tissue about 2 mm. in thickness. Probes passed through these two openings immediately entered into a globular aneurysm, which measured 6 cm. in diameter and laid outside the aorta in the right one half of the coronary sulcus.

The walls of this aneurysm measured 2 to 4 mm. in thickness, and on its inner surface contained organized and laminated blood clot. The coronary artery was lost in the aneurysm and a very small descending branch was dissected out, running in the posterior interventricular groove. The aneurysm had ruptured directly into the pericardial cavity through a linear break about 1.5 cm. in length.

The left or anterior coronary artery could be easily traced; it gave off a circumflex and descending branch, the latter descending in the anterior interventricular groove. The artery showed a few yellowish placques and was placed rather deep in the heart muscle on account of the old pericarditis.

As regards the etiology of this lesion, we have undoubted gross evidence of a luetic mesaortitis. Examinations for spirochetes were not made; but a negative result in this case would not influence the gross diagnosis, as it has been my experience and that of others (Aschoff, Crowell, Brown, Larkin, and Symmers) that positive findings of treponema in aortic aneurysms are unusual.

It is quite common to find at autopsy that the right anterior aortic sinus gives off a small accessory branch for the right coronary artery; this I believe accounts for the two openings encountered in this specimen.

The specimen is of unusual interest on account of the size of the aneurysm; most of the hitherto reported cases were small miliary aneurysms occurring in diseased coronaries the seat of luetic lesions and rarely reaching a size greater than that of a pigeon's egg.

RECURRENT SQUAMOUS CELL EPITHELIOMA OF TONGUE WITH WIDESPREAD AND UNUSUAL METASTASIS

B. M. VANCE, M.D.

(Pathologist to the Second Surgical Division, Bellevue Hospital, Charles Norris, M.D., Director)

The following case is a remarkable example of the extensive and somewhat rare metastases which resulted from a recurrent squamous-cell epithelioma of the tongue.

The patient, W. H., male, age 41 years, was admitted to the Second Surgical Division, Bellevue Hospital, complaining of painful swellings on both sides of his neck. His history disclosed that he had had a chancre twenty years before and that he had received active antisyphilitic treatment at different times in the past. Three years ago he was operated on for some condition on the dorsum of his tongue, the nature of which could not be determined. Three months ago a swelling appeared on the right side of his neck, and two months later a similar tumor started to develop on the left side. Both of these have steadily increased in size and have become inflamed and tender. One week before admission, a private physician incised the swelling on the right side under the impression that it was an abscess, but without result. The patient said that he had lost no weight, but was suffering from weakness and loss of appetite.

Physical examination on admission showed a small, poorly nourished and poorly developed, middle-aged male. He was somewhat anemic and his skin showed a peculiar muddy pallor. There was a firm, indurated mass, three inches in diameter, on the right side of the neck just under the angle of the jaw, and a similar swelling (two and a half inches in diameter) on the left side in the lymphatic tissues of the anterior cervical region. In the midline of the dorsum of the tongue, there was a longitudinal scar, the result of the previous operation.

Radiographic examination of the chest disclosed enlarged bronchial lymph nodes, especially on the left side.

The Wassermann reaction was strongly positive.

The lymph-nodes on the left side of the neck were excised for the purpose of diagnosis, and histological examination showed that the tumor was a squamous-cell epithelioma characterized by much cornification and pearl formation. The patient remained in the ward without change until the twentieth day after operation, when his heart suddenly began to fail and he died fifteen hours after the onset of symptoms.

At autopsy, neoplastic nodules were found in the lungs, liver, both kidneys, right adrenal, bronchial lymph-nodes, twelfth dorsal and first lumbar vertebræ, right bronchus, right lobe of thyroid, and myocardium. On microscopic examination, these nodules were found to be the same type of squamous-cell epithelioma that was demonstrated in the cervical node removed at operation.

The tumor in the heart deserves more detailed description, as it probably had a close connection with the patient's sudden exitus. It was a cream-colored, irregularly ovoid nodule, about three centimeters in diameter, embedded in the myocardium of the left ventricle, just beneath and alongside of the middle portion of the coronary artery and vein. A histological examination of the cross section of the vessels in this region showed the artery uninvolved, but the coronary vein thrombosed, while some of its tributaries were filled with epitheliomatous tissue. It is evident that the metastasis in the muscle had invaded the smaller veins, causing a thrombosis which, gradually extending to the larger trunk, proved too severe an embarrassment to an already overburdened heart.

The scar on the dorsum of the tongue showed no evidence of epitheliomatous growth, and was composed entirely of cicatricial fibrous tissue. There is good reason to believe, however, that the tongue was the probable primary site of the epithelioma. The evidence at hand, while not conclusive, is suggestive and nothing could be found that would indicate a different point of origin for a neoplasm of this type.

THE INFLUENCE OF SPLEEN UPON TUMOR GROWTH IN THE CHICK EMBRYO

HOLLAND N. STEVENSON, M.D.

(From Columbia University, George Crocker Special Research Fund, F. C. Wood, Director)

During the course of an investigation carried out according to the method for growing tumors in the chick embryo published by Murphy,[1] an experiment was undertaken to determine whether or not adult chicken spleen would inhibit the growth of a tumor other than the one which this author found would not grow under these conditions. This inquiry seemed warranted because Murphy's observations contradict the general law of tumor immunity, which is that immunity to a tumor can not be induced by tissue from a species of animal different from that to which the tumor belongs.

It was found in this experiment that the tumor used would grow freely despite the presence of growing chicken spleen on the allantois of the embryo. This was in such direct contradiction to the results reported by Murphy, that the work was repeated on a large scale.

The following tumors were used: The Ehrlich mouse sarcoma, the Jensen rat sarcoma, the Crocker tumors rat sarcoma 8, mouse carcinoma 11, mouse sarcoma 180, and the Imperial Cancer Research Fund mouse carcinoma 63. In each series the same dosage of tumor and also of spleen was used. The inoculations were made on the seventh day of incubation, the spleen and tumor being placed on the allantois at the same time. The grafts were removed and examined on the seventeenth or eighteenth day of incubation.

It was found in each instance that the tumors grew well and without any apparent inhibition. It seemed to make no difference whether the spleen was at a distance from the tumor graft or in close proximity to it. No unaccountable influence of

[1] *Jour. Exper. Med.*, 1914, xix, 181.

the spleen upon the size to which a tumor attained or upon the percentage of takes was noted. The tumors were all actively growing and showed numerous mitotic figures. The only apparent difference between the tumors growing under the influence of spleen and the control tumors was in the amount of cellular reaction. This was undoubtedly increased when spleen was present, but the cells comprising it were almost all myeloid cells.

The recent observations of Danchakoff[2] upon the influence of splenic grafts on the allantois upon the hematopoietic system of the chick embryo have an interesting bearing upon these results. She found that the spleen graft stimulated the production of myeloid cells by the mesenchyme tissue throughout the entire body of the chick, including the membranes.

In the control tumor grafts the reaction of the wandering cells varies from one that is quite intense to one that is barely noticeable. The increased cellular reaction around tumors growing in the presence of spleen is undoubtedly the result of the general hematopoietic stimulation. Its localization around the tumor graft indicates that where the tumor has the power to induce a certain amount of reaction in the normal chick, in the chick stimulated by spleen, this reaction is greatly magnified.

Details of the experiments will be published elsewhere.

Discussion:

Dr. Murphy: We used for our work the sarcoma of the rat, the old Jensen sarcoma. It had been thoroughly studied, and for that reason was selected. We used about 3,000 embryos in the experiments with uniform results with this tumor. I did not try other tumors. It is rather interesting to see that there are variations apparently with different tumors. Tumor No. 180 interests me particularly, because we have studied this tumor in our laboratory, and have been unable to get any immunity to it in mice. We have tried all the methods that have been established for the production of immunity, including the *x*-ray, and have failed.

Dr. Pappenheimer: It is very interesting that there should be individual variations as regards the immunity produced by spleen implantation.

Dr. Murphy: Of course there are individual variations in all these rat and mice tumors. That is why one worker repeats another one's experi-

[2] *Am. Jour. Anat.*, 1916, xx, 255.

ments and gets entirely different results. A variation of this kind between the transplantable tumors does not alter the general principle involved. The question of dosage comes in, and I think my spleen grafts were considerably larger than the ones Dr. Stevenson used.

Dr. Pappenheimer: Were there any experiments made with carcinoma?

Dr. Murphy: No, I have transplanted carcinoma to chick embryos, but I did none of the spleen experiments with carcinoma.

Dr. Stevenson: Crocker Fund tumor No. 11 is a carcinoma. Regarding the variations shown by different tumors in their susceptibility to immunizing agents, as brought out by Dr. Murphy in his discussion, it was fortunate that we used tumors of two distinct types. One of these (No. 180) it has so far proved impossible to immunize against in the mouse, while 100 per cent. of animals can, by suitable means, be protected against the other (No. 11). It is particularly to be noted, however, that both of these tumors, one entirely insusceptible to all attempts at immunization and the other readily yielding, do not differ from one another in their growth in the egg. Both grow equally well in combination with splenic tissue.

A CASE OF UMBILICAL POLYP

B. T. TERRY, M.D.

(*From the Brooklyn Laboratories of Pathology, Kings County Hospital, Brooklyn, N. Y.*)

A short time ago a small mass of tissue which had been removed from the umbilical region of a patient was received for diagnosis at the Brooklyn Laboratories of Pathology. This tissue presented a most unusual microscopical picture. In the center was a polyp covered externally by intestinal mucosa. Adjoining this polyp on each side were skin and subcutaneous tissue. For permission to report this case and for the hospital notes supplied me, I desire to thank Dr. Schall, chief of the surgical service at Cumberland Street Hospital, and Dr. H. R. Tarbox, the operating surgeon.

The patient was an Italian girl of twelve who had entered Cumberland Street Hospital seeking advice and relief on account of a small growth at the navel. This growth seems to have inconvenienced her chiefly because it bled readily and freely.

History: The family and personal history were negative. According to the child's mother, however, the trouble on account of which the patient entered the hospital dated back almost to the day of her birth. As soon as the umbilical cord came away,

FIG. 1. Umbilical polyp.

a small, reddish, rounded mass was seen at the navel. This mass had not increased in size, nor had it been painful, but it never healed and it bled with great readiness. In recent months, the mother thought, the tendency to bleed had increased markedly.

The physical examination showed a well-developed Italian girl of twelve. Her navel fossa was filled by a red, granular, circular polyp that measured about 1.75 cm. in diameter and projected about 0.5 cm. above the surrounding skin. The polyp was probed to see if there was a communication with the peritoneal cavity. No communication was found, but the polyp bled freely on being touched.

There were no evidences of umbilical hernia, and no weakness of the rectus muscle. The von Pirquet and Wassermann reactions were negative.

Treatment: A four-inch elliptical incision was made around the umbilicus and the polyp was carefully dissected down to the rectus. On removing the polyp, a small duct-like opening passing into the abdominal cavity was observed. Into this opening a probe was inserted for a distance of about two and one half inches, but no obstruction was encountered. The thin-wall duct was then tied off and after the stump was touched with a cautery, it was tucked away in the sheath of the rectus muscle, the abdominal wound was closed, and the patient made a satisfactory and uneventful recovery.

The specimen removed at operation was fixed in formalin and sent to the laboratories, where it was found to consist in the main of skin measuring 2×2.5 cm. Projecting from this skin was a mass of grayish tissue 0.7 cm. in diameter. On section this gray mass appeared to have a rich blood supply. To give a better idea of the specimen a careful drawing of the polyp is submitted (see Fig. 1). Fig. 1 gives a good idea of the general relations and appearance of the polyp, but the finer points can probably not be made out from this alone. The polyp consisted of a central core of connective tissue and smooth muscle and was covered externally by a mucous membrane resembling that of the small intestine. At the sides of the polyp this mucosa abruptly joined the skin of the abdomen. Along the protected lateral border of the polyp could be seen villi and glands of Lieberkühn. The villi were missing from the border exposed to trauma, and here with higher powers of the microscope, evi-

dences of acute and chronic inflammatory changes could be seen. In the tunica propria of the mucosa could be seen numerous dilated, hyperemic, thin-walled blood-vessels and an abundant infiltration of plasma cells and polymorphonuclear leucocytes.

The glands of the mucosa contained many goblet cells and in the better protected places a distinct cuticular border could be made out. Just external to the mucosa in the portion most exposed to trauma was found a bloody exudate containing a number of polymorphonuclear leucocytes, and external to this exudate and covering it was a thin layer of cells with long thin nuclei, this layer apparently being only one to three cells thick. It had seemingly been produced in a vain attempt to cover over the polyp and protect it from trauma. At places below the tunica propria, muscle could be seen probably representing the muscularis mucosa, and beneath this was a core of connective tissue through which ran thick bundles of smooth muscle. Dilated blood-vessels and lymphatics were present in the tunica propria, and, also, in the core of the polyp. Solitary lymph-nodes were also present in the mucosa. The skin of the abdomen was thin but normal in appearance.

Frequence: Cases of umbilical polyp are rare, and should be distinguished from the fairly frequent cases of granulation tissue. Granulation tissue at the navel is usually found only during the first few weeks after birth, is soft, dull red or pink in color, shows a purulent secretion, and usually disappears either spontaneously or after the use of astringents. Umbilical polyps, on the other hand, may persist for years, are of a brighter red color, are firmer, and are more resistant. Unless they have become infected, they secrete mucus. If infected, they secrete mucopus. They are usually not affected by astringents and persist until they are removed surgically.[1]

Demonstration: A section of the polyp from the patient in Cumberland Street Hospital was projected, as were also pictures

[1] For the literature, embryology, complications, and treatment of cases of umbilical polyp, Thomas Stephen Cullen's recent (1916) book, *The Umbilicus and its Diseases,* W. B. Saunders Co., may be consulted.

which explained embryologically the development of umbilical polyps from a persistent vitelline or omphalomesenteric duct. Other pictures showed that although the girl will probably never again have bleeding from the umbilicus, she may in the future have intestinal obstruction, due to persistence of the vitelline duct in whole or in part in the abdominal cavity. In view of these possibilities, before the patient left the hospital, her parents were warned to bring her back at once should she ever show any signs of serious abdominal trouble.

A CASE OF ANTHRAX, WITH DEMONSTRATION OF SLIDES

B. T. TERRY, M.D., AND MORRIS B. BEECROFT, M.D.

(From the Brooklyn Laboratories of Pathology, Kings County Hospital, Brooklyn, N. Y.)

The case here reported is apparently the only one of anthrax that has been admitted to Kings County Hospital in the last four or more years. It is presented for the following reasons:

1. It seems very desirable that cases of this kind be recognized early in order that prompt treatment may be instituted. The reporting of cases may direct attention to the disease and aid in its diagnosis.

2. The *Bacillus anthracis* is so large that it can be readily demonstrated with a small portable projectoscope, such as we have brought with us to-night, provided the distance to the screen is not too great.

The patient entered the hospital in October in the service of Dr. J. B. Bogart, to whom we desire to express here our thanks for permission to report the case. She was a widow, twenty-seven years old; the chief complaint was pain and swelling of the face.

History: Her family history and personal history were negative except that for the past year she had worked in a shaving

brush factory in New York City. She had two children who are said to be living and well.

Present Illness: Two days before her admission to the hospital, the patient noticed that the left side of her face had begun to swell. At first the swelling was painless. It increased so rapidly in extent, however, that she soon became alarmed and entered the hospital for treatment.

The Progress of the Case: As the tentative laboratory diagnosis on the day after admission was anthrax, the patient was seen that evening by one of us and her face was found to be greatly distorted. The lips were so swollen and edematous that she could not close her mouth. In the open mouth was a purulent looking exudate that was rather profuse and had a foul odor. Every few minutes a nurse swabbed the mouth out with a sponge on the end of a stick. The whole face appeared terribly swollen, the left side being more affected than the right. The left eye was completely closed but the right eye could still be seen through the narrow slit-like opening between the thick edematous upper and lower lids. The patient was conscious and could still make herself understood; she said she was in great pain.

The next day, the second after admission, the patient was apparently weaker and the swelling of her face was, if anything, greater than before. Serum was ordered by the hospital from Washington, and was received early in the morning of the third day after admission, but the patient was then very weak and at times irrational. The intravenous injection of the forty c.c. of serum was begun as soon as possible. During the injection, the patient was irrational, talked continuously, and tried to get out of bed. She was so ill that stimulants were administered and external heat was applied.

In spite of these measures, however, her pulse gradually became imperceptible and her extremities cyanotic and cold. Shortly afterwards the patient ceased to breathe.

At the time the patient died, the first guinea-pig we had inoculated was still alive, and the laboratory's tentative diagnosis of anthrax was not accepted by the coroner's physician. For

this reason, no autopsy was permitted. Shortly before the death of the patient, when a fatal termination seemed inevitable and imminent, a small bit of tissue was ordered removed from the upper lip on the left side, in order that the diagnosis might be established beyond doubt. This bit of tissue was obtained and was divided in two parts. One of these was introduced subcutaneously into a second guinea-pig, and the other piece was fixed, embedded, and subsequently studied histologically.

Laboratory Work: This was done by Dr. Beecroft, who, on the day after the patient's admission, was requested to see her and to determine, if possible, the nature of the infection. The lesion on the upper lip was cleaned and an incision was made in it in order that fluid from the inside might be examined. From this fluid smears and cultures were made and a little of the fluid, after being collected in a sterile pipette, was diluted and injected intraperitoneally into a guinea-pig.

The smears showed a large Gram-positive bacillus and several other Gram-positive cocci, including a staphylococcus, a streptococcus, and a diplococcus.

Cultures made in broth and agar showed the same organisms. On plating, the large Gram-positive bacillus was obtained in pure culture. This grew aerobically readily, and rapidly on ordinary culture media. It formed long chains and spores developed after twenty-four to forty-eight hours. Agar colonies transferred to slides with as little disturbance as possible and then stained showed typical "medusa head" appearances. The organism seemed absolutely non-motile even when precautions were taken to test young cultures in hanging drops on a warm stage. The bacillus was so large that it was readily seen with a No. 3 Leitz objective and a No. 4 eye piece. It measured about 6 to 10 micra long and 1 to 2 micra in thickness.

Pathogenicity: The first guinea-pig was inoculated intraperitoneally with diluted lip fluid which, according to cultures and smears, contained a Gram-positive bacillus and several Gram-positive cocci. The guinea-pig became sick promptly, but did not die until about the seventy-second hour. At autopsy, smears

showed the Gram-positive bacillus apparently in pure culture in the peritoneal exudate, heart blood, lungs, spleen, kidneys, and liver. In the case of the heart blood, the spleen, and the liver, cultures were made and in each instance the Gram-positive bacillus grew out apparently in pure culture.

Studied histologically, sections of the heart, lungs, spleen, kidneys, and liver showed the Gram-positive bacillus present in very large numbers in the blood-vessels of these organs and in the exudate on the surface of the liver.

The second guinea-pig, that was inoculated subcutaneously with a small piece of the lip of the young woman, died in about thirty-six hours. Except that no peritonitis was found in the autopsy of this animal, the autopsy findings were practically the same as in the case of the first guinea-pig. The Gram-positive bacillus was seemingly present everywhere in large numbers and in pure culture. This was indicated by smears and cultures made from its organs and by a histological study of the organs of this guinea-pig.

Patient's Lip: On examining histologically the bit of tissue removed from the young woman's lip, the same large Gram-positive bacillus was found in large numbers superficially, and in smaller numbers in the depths of the tissue. The organisms were seemingly present in greatest number in the exudate on the surface of the lip.

Diagnosis: The diagnosis in this case seems reasonably certain. In the shaving brush factory, the patient had worked with imported hair. The infection of the lip was attended by great local edema and ran a rapid course, terminating fatally on the fifth day after the swelling was first noted. From the lesion in the lip, a large spore-bearing, non-motile, Gram-positive bacillus was isolated. This bacillus grew aerobically and readily in long chains on ordinary media, and the ends of the bacillus were square. Fluid and tissue from the lip killed two guinea-pigs, and at the autopsy of the guinea-pigs the large Gram-positive bacillus was seen in all organs studied and was recovered in pure culture from the heart blood, the spleen, and the liver of each guinea-pig.

Demonstration: Slides were demonstrated to show the appearance of a colony grown on agar and transferred as a whole to a slide and stained. Another slide showed the organisms on the surface of the young woman's lip and in its depths. Still others showed the bacilli present in the organs of the guinea-pigs killed by the bacilli.

Effect of Case: After our guinea-pigs died, the New York City Health Department took a lively interest in the case, and we are informed that the factory where the young woman worked has been thoroughly investigated and strict orders have been issued to sterilize thoroughly all imported hair before turning it over to the workers.

UTERUS SHOWING TWO TYPES OF MALIGNANCY

HARRY S. BERNSTEIN, M.D.

(From the Bender Hygienic Laboratory, Albany, N. Y., and the Laboratory of Pathology and Bacteriology of the Rhode Island State Board of Health, Providence, R. I.)

An adenocarcinoma of the uterus complicated by a leiomyosarcomatous process which involved portions of its musculature is of unusual occurrence. The following analysis of 627 panhysterectomies emphasizes the extreme rarity of the condition; of that total, 283 uteri showed changes secondary to disease of the appendages. The total included, also, 290 specimens of leiomyomata, 6 of which showed, in addition, the presence of adenocarcinoma, 3 of epidermoid carcinoma, and one of tuberculosis. There were, also, four specimens of malignant leiomyomata, two of which exhibited the so-called "perithelial angiosarcomatous" type of growth. The remainder consisted of fourteen adenomyomata, eleven adenocarcinomata, twenty-four epidermoid carcinomata, and one combined adenocarcinoma and leiomyosarcoma. Our statistics indicate, therefore, the occurrence of the last named form of malignancy in a little more than one tenth of one per cent. of cases in which a panhysterectomy has been performed.

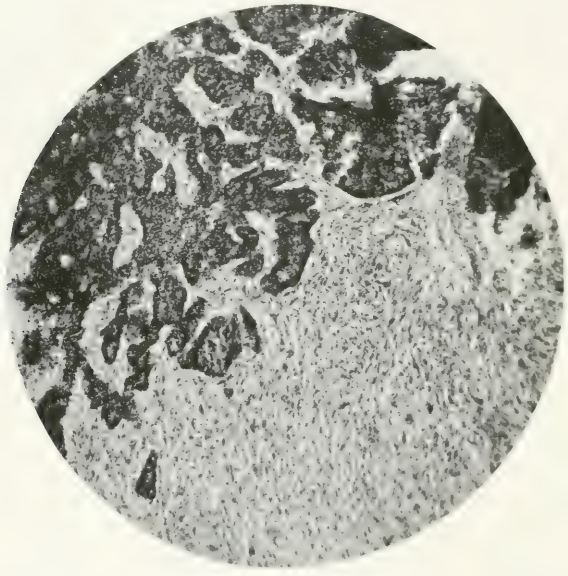
The specimen in question was removed from a woman, fifty-two years of age. The relevant points in the history embrace the following facts: Menses began at the age of fifteen and continued until she became thirty-four. She was married at nineteen and gave birth to twelve children. There were no miscarriages. At the age of forty-eight, fourteen years after the menopause, uterine bleeding reappeared. The flowing was more or less constant and the loss of blood was considerable in amount. Two years after the onset of her ailment, a curettage was performed. The microscopic examination of the curettings showed adenocarcinoma. Surgical intervention, however, did not take place until two years thereafter. During the five weeks prior to the operation, she suffered from intense pain low down on the left side. The flowing had meanwhile been continuous, the discharge consisting of blood-clot and of a grayish material which possessed a foul odor. The appetite was poor, and there was a coincident loss of weight.

On physical examination, a mass, apparently the enlarged uterus, could be felt through the anterior abdominal wall. There was marked tenderness and slight rigidity over the left inguinal ring. Bimanual examination showed a slightly patulous cervix. The external os was hard and indurated. There was no apparent evidence of an evertive growth. It appeared as if the uterus were bound down to the surrounding tissues. The fundus was apparently greatly enlarged and boggy.

At operation, the tissues proved to be so soft that the uterus tore off from the cervical neck. The cervix was thereupon removed separately. In the left iliac fossa, there was a portion of the omentum which contained a firm, irregularly defined nodule, about the size of a billiard ball.

The removed uterus, almost globular in form, measured 8 cm. in length and 12 cm. in its greatest width. The serosa was smooth. The consistence was diminished and the entire specimen felt as if it were a fluctuating mass. On section, the uterine cavity was much enlarged, and was filled with a large amount of grayish, friable, foul-smelling material, which was mixed with

blood-clot. On serial section, it was evident that the grayish material infiltrated the uterine musculature and gradually blended with it. On removal of the contents from what appeared to be the uterine cavity, the entire lining seemed involved and presented a large ulcerating surface. The uterine wall varied in width,

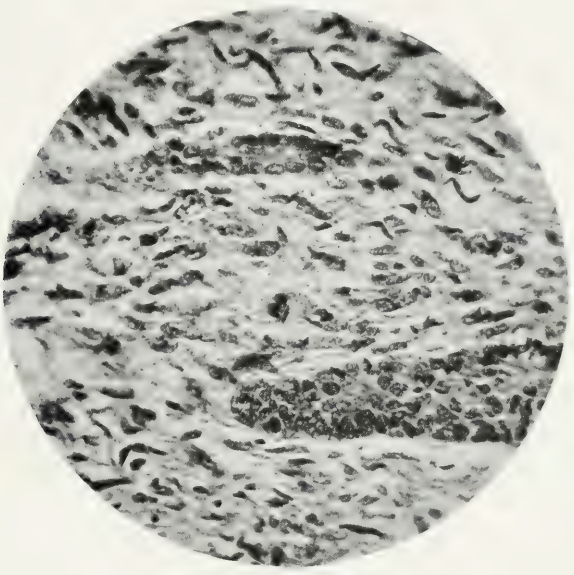


Low power: Showing the relation of the two types of malignancy.

averaging 4 cm. The attached Fallopian tubes were slightly tortuous and thickened. The ovaries were small and hard. The mass of fat of omental origin contained a nodule of grayish, soft tissue. The cut surface was elevated and presented a moist and granular appearance.

Microscopic sections taken from different parts of the uterine wall showed the following features: An atypical glandular epithelium infiltrated smooth muscular tissue. In some areas, a tubular gland, lined with a simple ciliated columnar epithelium, dissected bundles of smooth muscle fibers. In other areas, the epithelial cells were congregated in large numbers and were connected with similar masses by narrow chains of epithelial

cells. In such accumulations, the outermost layers of cells were more or less cylindrical. The cells gradually became circular in outline towards the center. Here and there, within these masses, there were attempts at gland formation. The lumina of the glands were bounded by a ciliated cylindrical epithelium or by a



High power of same.

non-ciliated cuboidal epithelium. Extensive necrosis was present, both of the glandular portion and of the uterine wall. Mitotic figures were numerous and occasionally direct cell division occurred. The smooth muscle tissue, surrounding the invading tumor cells, appeared normal, save for edema and infiltration with plasma cells. In a few of the sections, however, the smooth muscle cells showed a marked deviation from the normal. The arrangement of the cells was not unlike that of the layers of normal cells seen elsewhere in the specimen. The cells moreover, larger than normal, showed every gradation from an elongated spindle shape to spherical, and the nuclei likewise varied from an ovoid to a circular outline. The chromatic material in

the latter consisted of coarse granules. Stained with phosphotungstic acid hematoxylin, the spindle cells showed the presence of delicate myoglia fibrils: and scattered between the cells were many bundles of collagen fibrils. Mitotic figures were abundant. It was evident, also, that cell multiplication was more active in some areas than in others. It thus appeared that the adenocarcinomatous growth, previously described, was invading a region of the uterine wall, the cells of which were involved in a malignant process. The appendages were not remarkable. The lymph-node in the omental fat showed invasion with the adenomatous type of malignancy.

It is to be emphasized that the sarcomatous process involved only a portion of the anterior wall of the uterus. Its origin must of necessity remain a matter of conjecture. According to the prevailing opinion, uterine sarcoma may arise from the endometrium, from a myoma, or from the uterine wall. The extensive necrosis of the contents of the uterine cavity and the absence of any definitely circumscribed tumor within the uterine wall restricts the possible source of the sarcoma to the uterine musculature itself. Moreover, the freedom of the sarcomatous process from any necrosis may indicate that its inception was more recent than that of the extensive adenocarcinoma. The panhysterectomy, it will be recalled, was performed four years after the onset of symptoms. The carcinoma was, therefore, at least of four years' duration. The patient, moreover, had gone through twelve pregnancies; and this fact may account for an irritability on the part of the uterine musculature. It is not unreasonable to conclude, therefore, that the uterus in question shows a somewhat localized leiomyosarcomatous reaction to an adenocarcinoma of long standing.

A SUSPECTED CASE OF MERCURIC CHLORIDE POISONING

A. O. GETTLER, PH.D., AND A. V. ST. GEORGE, M.D.

(From the Pathological Department of Bellevue Hospital, Charles Norris, M.D., Director)

The following case is reported because of the difficulty of properly correlating the clinical history and the interesting pathologic findings with the chemical analyses performed during life and after death.

L. C. (No. 5355), female, single, age 30 years, seamstress, was admitted to ward A-3 and transferred after a few hours to ward 34, Bellevue Hospital (Dr. M. S. Gregory, director of psychopathic service, whom we desire to thank for permission to abstract the clinical records), on January 31, 1917, with a history of having taken thirty tablets of bichloride of mercury two days before admission. She vomited constantly a good deal of food and mucoid material containing blood. She complained of continuous pain in the abdomen and had a moderately bloody diarrhea. On February 6 she vomited blood-clot following passage of stomach tube; and on February 8, again, vomited blood-clots and also dark red blood on February 9. The bowels were loose, never more than three times a day. On February 8, she was given high colon irrigation; and the returns showed dark, almost black, feces. Horse serum was administered to stop the bleeding. The patient was stuporous part of the time and, finally, became comatose and died on February 13, 1917.

Her brother gave the following history: The patient had had an attack of insanity three years before while in Italy. For the last two months she had been morose and depressed, saying that she was forsaken by everybody and that people wanted to do her harm. On January 29, she went to confession; but the priest said that he could not hear her confession because he did not understand Italian. This the patient interpreted as meaning that God, also, had forsaken her and she, thereupon, took the tablets of

mercury. There were no tablets of mercury, or a container therefor, or ammonia water or any other corrosive poison found in the house, and the druggist from whom she claimed to have bought them denied the sale.

Physical examination was unimportant. Catheterization on February 8 gave 16 ounces of clear urine, sp. gr. 1.015, which contained a marked trace of albumin, no sugar, and no casts. She continued to urinate as before, the daily quantity increasing each day, but the daily average was 6 to 8 ounces.

On admission the temperature was 97.8, and it remained subnormal except for a rise on February 5 to 101° which lasted for a day. The pulse was 70 to 120, and respirations, 12 to 40.

The laboratory examinations resulted as follows:

1. Jan. 31 (7687). Examination of vomitus for mercury, negative.[1]

2. Feb. 9. Blood culture negative. Wassermann negative.

3. Feb. 13 (7859). Test for mercury in feces, negative.

4. Feb. 14 (7946). Examination of kidney for mercury, negative.

Examination of intestinal contents for arsenic and mercury, negative. Examination for ammonia showed free ammonia present.

Clinical Diagnosis: Mercuric chloride poisoning.

Autopsy (by Dr. Benjamin Schwartz, coroner's physician, whom we wish to thank for permission to use his report):

Anatomical Diagnosis: Acute esophagitis, acute gastritis, and acute enteritis with intestinal and gastric hemorrhages (corrosive poison). Acute parenchymatous nephritis. Ulcers of colon. Acute parenchymatous degeneration of the liver. Acute suppurative pericarditis. Pulmonary tuberculosis, apical, healed.

[1] Reinsch's Test. The organ is decomposed with potassium chlorate and hydrochloric acid, until the liquid is slightly yellow; the excess of chlorine is boiled off; the solution is neutralized with ammonia water, and then slightly acidified with hydrochloric acid. Clean copper wire is inserted, and allowed to stand over night. The wire is then washed with water, alcohol, and ether, and the mercury either sublimed on gold leaf, according to Vogel's technique, or allowed to deposit on a clean glass tube.

Status lymphaticus, recessive. Infantile uterus. Patent foramen ovale. Patent thyroglossal duct. Malformation of chest. Cysts of left parovariun.

Kidneys: Together weighed 400 gm. They were large and fairly firm, and were surrounded by the normal amount of fat. The capsule stripped with ease, leaving a smooth, pale, pinkish gray surface. On section, the cortex was wider than normal, measured 1 cm. in thickness, and bulged beyond the cut edge of the capsule. The cortex was quite gray in color and streaked by widely separated but injected vascular channels. The cut surface was shiny, and the glomeruli could be readily seen. The pyramids were distinctly violaceous in color. The vessels were normal. The mucous membrane of the pelvis was injected. The ureters were normal. The bladder mucous membrane was pale, but showed no other alteration.

Gastrointestinal Tract: The base of the tongue was markedly swollen and edematous. The foramen cæcum was patent and admitted the point of a probe for a distance of 1 cm. The mucous membrane of the upper half of the esophagus was swollen and gray in color. Over the whole was a delicate, gray, somewhat tenacious membrane. The mucous membrane of the lower 10 cm. of the esophagus was intensely congested and swollen. In the lower 2 cm., the mucous membrane was soft, swollen, and dark brown. The stomach contained a brownish red mucous material, in which there were a few blood clots. The inner surface of the stomach was bluish brown in color, and was covered by a thick mucus. On removing this, the lining surface of the stomach was found to be greatly swollen and translucent, and over it were many spider-like and angular red areas. The rugæ were fairly prominent. There were no ulcerations. The small intestine contained the same kind of bloody material. The mucous membrane was, also, swollen, reddish brown, and covered with thick mucus. A distinct odor of ammonia was emitted by the intestinal contents, which were markedly alkaline to litmus paper. There were no ulcerations in this part of the intestinal tract. The mucous membrane of the large

intestine was, also, swollen and covered by bloody mucus. It was intensely red in patches and showed irregular circumferential streaks. In the ascending colon, there were many rounded, sharply circumscribed, and raised areas measuring about 1.5 cm. in diameter. These areas were fairly firm, a dirty grayish brown, and covered by slough.

Smears made from the pericardial sac showed numerous pus cells.

Microscopic examination of the kidney showed marked acute parenchymatous nephritis with considerable calcium deposits in the tubules, best shown by Von Kossa's (silver nitrate) stain. The esophagus, stomach, and intestine showed epithelial destruction and inflammatory reaction due to a corrosive poison. The colon showed marked diphtheritic inflammation with ulceration.

The negative chemical findings in contradistinction to the history of the ingestion of mercuric chloride and the typical pathologic picture of mercury poisoning (acute parenchymatous nephritis with calcium deposit in the tubules and the typical lesions in the colon) made us strongly suspect that there were some other factors involved. The clinical picture, also, as well as the course of the disease left considerable room for doubt.

The question as to how the free ammonia came into the stomach can not be definitely settled. The patient had not received aromatic spirits of ammonia just before death, nor in fact any medicinal preparation containing ammonia or one of its derivatives at any time during her stay in the hospital. It was thought that she might have taken some ammonia water or some cleansing preparation containing it while in the hospital. But none of these preparations are used or kept in the wards of the hospital. All toxicologists (1) and pharmacologists (2) are agreed that free ammonia remains but a relatively short time in the body, not longer than two days. To satisfy ourselves on this point, we gave a cat 10 c.c. of strong ammonia water. The cat immediately became acutely ill, and within five minutes vomited all her food and, within half an hour, blood stained mucus. She continued sick and, finally, five days later, was anesthetized and

killed (with ether). Autopsy showed a typical acute esophagitis, gastritis, and enteritis, but no other lesions. Section of the kidney showed a perfectly normal organ. The reaction of the gastric contents was acid. Chemical examination of the gastric and intestinal contents did not reveal the presence of free ammonia.

On the assumption that the patient might have taken a double salt of mercury, we administered some freshly prepared ammoniated mercury to two cats. To one of them we gave 10 gm. of the salt, and to another 50 gm. Both animals became acutely ill and within half an hour vomited a good deal of the salt administered. The one receiving the 10 gm. fully recovered, and at the end of 10 to 12 days was quite normal again. The cat receiving the 50 gm. appeared to be quite sick, became weaker and weaker, and died at the end of six days. On autopsy, the animal showed a normal esophagus and stomach, the latter with an alkaline reaction. The kidneys on gross examination presented the typical picture of mercury poisoning. The bladder was entirely empty and its wall was "dry." As far as could be ascertained the animal had had an anuria during the last few days of life. Chemical examination of feces and stomach contents was negative for mercury. Chemical examination of one kidney for mercury showed the presence of a very faint trace. Microscopic examination of the kidney showed an acute parenchymatous nephritis with marked fatty degeneration. It is, therefore, possible to produce a somewhat similar lesion with the ammoniated mercury, though this because of its practical insolubility must be given in relatively enormous quantities. And, again, we must not forget that although the kidney showed marked and extensive lesions, yet there were none of the calcium deposits present, nor were there the usual lesions in the colon.

Bismuth is known to produce lesions similar to those of mercury, but only after systemic absorption from an open wound, such as comes from the injection into an empyema cavity or bone sinus. The patient had none of these conditions. In order to feel somewhat more convinced that the druggist had not sold

her a poison of any kind we asked the Board of Pharmacy to cooperate with us, but it still could not be proved that she had purchased a poison from the pharmacy in question.

To sum up, then, we have a case the main features of which are an acute parenchymatous nephritis, an acute suppurative pericarditis, and changes in the gastrointestinal tract, suggesting the action of a corrosive poison. Should this case be regarded as one of low-grade nephritis in which an unknown corrosive poison containing an ammonia preparation had been taken, perhaps before admission to the hospital? In favor of this we have the absolutely negative chemical findings for mercury and other metallic poisons, the presence of ammonia, and the absence of anuria, with negative chemical and microscopic urinary findings. Against it, we have the positive history of mercuric chloride ingestion with the typical pathologic findings. The history may be eliminated because of the fact that the patient was insane. The pathologic findings as regards the kidney might be explained as one frequently finds calcium deposits in other conditions than mercury poisoning. Calcium has been found in various organs of the body without known significance. Rokitansky, v. Recklinghausen and Koster (3) and Pappenheimer (4) have found it in the heart muscle; and Norris (5) in cases of sepsis.

However, we have not explained our lesions in the gastrointestinal tract. Could she have taken all the tablets of mercury and immediately thereafter vomited, leaving only the eschars on the mucous membrane of the stomach and esophagus? Again, we have no adequate explanation for the presence of the kidney and colon lesions. The lesions in the former could not be produced without the absorption of the poison; and those in the latter, whether we accept Flexner and Sweet's (6) theory of the excretion of mercury in the colon, or Kaufmann's (7) theory of the thrombosis and localized areas of ischemia as the causative factors in the production of the ulcerations, also, presuppose an absorption of the poison. Further, from the experimental work of Burmeister and McNally (8) and Lieb (9) on dogs and

rabbits as to the rapidity of the absorption of the mercuric chloride, it seems unlikely, whether the patient did or did not vomit immediately after the ingestion of the tablets, that she would have vomited all of the poison. It is, also, fair to assume from the sensitiveness of the test for mercury, as determined experimentally by Vogel and Lee (10) and later demonstrated clinically by Lambert and Patterson (11), Gettler and Baker (12) and others, and by our own autopsy statistics, that the presence of even a trace of mercury would have been determined.

In conclusion, we are unable definitely to classify this case, except to say that possibly it corresponds to an idiopathic parenchymatous nephritis, with the so-called uremic necrotic colitis, a theory which is sustained by the presence of an acute suppurative pericarditis unassociated with bacteria.

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CASES OF TYPICAL AND ATYPICAL LYMPHOSARCOMA

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ABSTRACT

Kundrat and Paltauf were the first to differentiate lymphosarcoma from the group which in the older literature had been included under the term malignant lymphomata. Sternberg reviewed the subject in 1903 and added his own observations; according to this author the conception of Kundrat and Paltauf is briefly as follows: Lymphosarcoma is a tumor formation starting in a group of lymph-nodes and spreading thence to neighboring nodes or follicles; it spreads from region to region without ever exhibiting such general lymphoid involvement as leukemia or pseudoleukemia; sooner or later it invades the capsule and extends into the surrounding tissues. The metastases differ from those of ordinary neoplasms in that the intervening lymphoid tissues are involved. True metastases, which can be explained only through transfer by way of the blood stream, are rare and usually isolated. Blood-vessels are seldom invaded by lymphosarcoma, being usually only surrounded and narrowed. The commonest sites of origin are the lymph-nodes of the neck, mediastinum, and mesenteric and retroperitoneal regions; less frequent sites are the inguinal and axillary nodes. The affected groups form nodular, uneven masses, well limited in the beginning, but diffusely permeating the surroundings. They are mostly hard and grayish white, and show a homogeneous cut surface. The spleen is rarely attacked and the bone-marrow likewise. Histologically, the tumor is characterized by an irregular reticular framework with lymphoid cells in the meshes; the architecture of the gland is lost; follicles and medulla are no longer differentiated; and the capsule and surrounding tissues are diffusely infiltrated with tumor cells so that the separate lymph-nodes can be distinguished neither from one another nor from

the surrounding tissues. The cells resemble lymphocytes, but are larger, have more lightly staining nuclei, and have a scanty, often almost invisible, non-granular cytoplasm. There is no definite alteration of the blood picture.

The present communication deals with four cases taken from the autopsy records of the past five years at the Presbyterian Hospital. Two of these cases belong to the Kundrat-Paltauf group. In one of these the primary tumor was apparently about the rectum; from here it had extended to practically all the lymphoid tissue in the body and had extensively infiltrated the surrounding tissues. Foci were found in the spleen and bone-marrow, an unusual feature, though the occasional occurrence of such metastases has been recognized. In the second case, the primary growth was evidently in the lymph-nodes about the aorta. Its atypical features were the invasion of the spinal meninges, giving a clinical picture dominated by spinal-cord symptoms, and the presence of discrete nodules in the liver.

The third and fourth cases were more atypical than the other two and, hence, more difficult to classify. The similarity between the two cases was very striking in many particulars. Thus, the patients were about the same age; the disease ran its course in about the same time; the first lymphatic involvement noticed was in the groin; a marked enlargement of all the superficial lymph-nodes followed in a few months; and both cases showed enlargement of the liver and great enlargement of the spleen, as well as nodular masses in the lower abdomen. In their pathological anatomy, also, the two cases were very similar. Both exhibited general lymphatic enlargement; a tendency of the enlarged nodes to become fused together into large masses, without, however, diffusely invading their surroundings, as was the condition in the other two cases; a great enlargement of the spleen from the presence of discrete tumor nodules closely crowded together; involvement of the bronchi, pulmonary vessels, and pleura in characteristic manner; the absence of any change in the lymphoid tissues of the intestine; and, finally, the presence of discrete foci of tumor cells in the bone-marrow with-

out the presence of Bence-Jones protein in the urine. All these features were present in both cases. One of them did, however, show discrete nodular metastases in the liver and urinary bladder, while the other did not.

The resemblance between the two is further borne out by the histological picture. The structure of the tumor tissue and the size, shape, and staining affinities of the predominating cells were much the same in each. Case 4, however, had many more of the very large cells and these, moreover, were larger and had more darkly staining nuclei than the large cells in case 3.

When we try to place these two cases in any of the recognized groups of primary enlargement of the lymphoid tissues, difficulties are encountered at once. From the Kundrat lymphosarcoma they are distinguished by the following characteristics: (1) The early involvement of the lymph-nodes throughout the body, in a manner which does not suggest a regional extension. (2) The formation in great numbers of discrete nodular metastases in the liver (case 3) and spleen (both cases). (3) The much less marked tendency to grow out into the surrounding tissues, but rather to form large masses of nodes adherent to one another. (4) The very different histological picture: the greater average size of the cells and the variety in their size and shape, the more vesicular character of the nuclei, and the presence of very large cells which in one case had an appearance suggesting megalokaryocytes.

From pseudoleukemia or aleukocythemic lymphatic leukemia these cases were readily distinguished by the type of splenic enlargement, the nodular metastases, and the different histological picture, these cases having cells bearing no resemblance to the lymphocyte. From the leukosarcoma of Sternberg, assuming that there may be an aleukemic stage or form of this condition, the cases described may be distinguished: (1) By the fact that in leukosarcoma the splenic enlargement is due to a diffuse infiltration of the whole spleen by the abnormal cells and not to the presence of clusters of discrete nodules. (2) The liver is affected in leukosarcoma in the same way as in leukemia, that is,

by infiltrations. (3) By the absence in leukosarcoma of the very large cells found in the two cases described.

It is hardly necessary to point out that the histological picture alone of Hodgkin's disease is sufficiently characteristic to make it unnecessary to indicate other points of differentiation.

In conclusion, the four cases included in this preliminary report fall into two groups. The first two cases have enough in common with the Kundrat-Paltauf lymphosarcoma to be classed as such. The other two cases differ so essentially from lymphosarcoma, pseudoleukemia, and leukosarcoma, that to place them in any of these groups seems unjustifiable.

This preliminary communication in its unabbreviated form, including blood counts and illustrations, will appear in a forthcoming number of the *Journal of Cancer Research.*

Discussion:

Dr. Norris: I think we have all seen at autopsy cases that on section were hard to classify. In this country, if the lesions do not show the typical changes described by Dorothy Reed, most pathologists refuse to call the condition Hodgkin's disease, and use, instead, the term "lymphosarcoma." I prefer to follow the German school, and give them the title "malignant lymphoma." I have seen in Bellevue Hospital a number of cases resembling Dr. Mackenzie's, one only last week which was very similar to his first case. In this we made a diagnosis of Hodgkin's on account of the enlarged retroperitoneal lymph-nodes and spleen.

EXPERIMENTS ON THE RESISTANCE OF CANCER IMMUNE ANIMALS TO TUBERCULOSIS

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Lewis and Margot observed that the spleens of mice experimentally infected with tuberculosis were enlarged. Paradoxically, they found that mice splenectomized about three weeks before the injection of tubercle bacilli lived longer than normal animals (1). Murphy and Lange then demonstrated that the lymphocytes in splenectomized animals, while decreased imme-

diately after the operation, showed a marked rise above the normal by the nineteenth to the twenty-first day. The experiments of Murphy and Ellis (2) showed that the enhanced resistance of previously splenectomized animals, as well as normal ones, could be destroyed by repeated small doses of *x*-ray. Morton (3) has shown that *x*-rayed guinea pigs are more susceptible to human bacilli than normal animals.

The experiments of Murphy and Morton (4) demonstrated that animals immunized against a transplantable mouse carcinoma by a previous inoculation with normal living mouse tissue cells develop a marked lymphocytosis which lasts several weeks. Using this method of increasing the lymphoid tissue, the following experiments were performed:

Experiment 1: Fifteen mice, immunized against, and then inoculated with, cancer, and ten controls were inoculated intraperitoneally with 3 mgm. of a culture of bovine tubercle bacilli. The cancer immune animals lived on an average of 47.7 days, while the lives of the normals averaged but 20.3 days.

Experiment 2: Fourteen mice, immunized against, and then inoculated with, cancer, and ten control mice were inoculated in the same way, with a more virulent strain of the same culture. The average life of the cancer immune animals after inoculation was 24.3 days, and that of the normals 14.7 days.

These experiments seem to indicate that animals effectually immunized against transplantable mouse carcinoma likewise showed an increased resistance to tuberculous infection. To prove this point further, the following experiments were performed:

Experiment 3: Thirty-eight mice were immunized and inoculated with cancer in the same way as those in experiments 1 and 2. Before the injection of the tubercle bacilli, half of the animals were exposed to seven small daily doses of *x*-ray. All were then injected intraperitoneally with 2 mgm. of the same culture of the bovine tubercle bacilli. The intact cancer immune animals lived on the average 32.5 days, while those *x*-rayed lived only 14.5 days.

Experiment 4: Ten mice, previously immunized against, and inoculated with, cancer, were given seven daily *x*-ray exposures and then, together with ten normal mice, were injected intraperitoneally with 3 mgm. of the bovine culture. The lives of the normal mice averaged 18.0 days, against 8.9 days for the *x*-rayed cancer immune animals.

DISCUSSION AND SUMMARY

Evidence that the lymphocyte is concerned in tuberculous immunity may be summed up as follows: (*a*) It is constantly associated with the pathological lesions. (*b*) Evidence is found that this cell is increased in the blood in resistant human cases and decreased in those rapidly progressing. (*c*) *X*-rayed animals have less resistance to the infection than intact ones. (*d*) Cancer immune animals with a high blood lymphocyte count are more resistant to experimental tuberculosis than are normal animals.

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Discussion:

Dr. Norris: I would call attention to the fact that Dr. L'Esperance, at the February meeting of the Society, reported that she had been able to lengthen the lives of tuberculous animals by inoculating them with typhoid bacilli. According to Jobling, there are a number of agents capable of increasing the resistance to tuberculosis.

Dr. Pappenheimer: I would like to ask Dr. Murphy what explanation he has for the susceptibility of lymphoid tissue to tuberculosis, and for the fact that children, in whom the lymphoid tissue is particularly abundant and well developed, show no immunity to tuberculosis.

Dr. Murphy: We are not prepared to explain a great many things in the development of resistance to the tubercle bacillus. We have only made the observation that in tuberculosis resistance seems to depend on a lymphocytic reaction. Tuberculous lymph-nodes are most common, but a high percentage apparently heal satisfactorily. As to why this should be we have no direct suggestion to offer. There are a great many possibilities. Certainly the evidence that the lymphocyte does have a function in tuberculosis is most suggestive, though not conclusive; and we hope, by further studies, to determine what part it plays in this resistance.

Dr. Pappenheimer: Is there any difference in the lymphocytic count between the animals rendered immune to cancer and the controls after both have been inoculated with tubercle bacilli?

Dr. Murphy: We did not make counts on these animals, because in making blood counts on mice the relative amount of blood lost is so great that it is apt to throw the whole experiment off.

Dr. Schwarz: Infants who have a relatively high lymphocytic count are not so susceptible to tuberculosis and rarely contract the disease in the first six months. In the second, third, or fourth year, the lymphocytes approach more nearly to the adult count. It has been shown that in tuberculosis in infants a high lymphocytic count gives a favorable prognosis.

PRIMARY CARCINOMA OF THE LIVER

Report of Two Cases

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While there are many cases of primary carcinoma of the liver recorded in the literature, in the experience of any one pathologist they are relatively rare. The two cases presented here are of interest in that they represent two types, one being a small-cell carcinoma derived from the gall-ducts, while the other is of the large-cell type derived from the liver cells.

The first case occurred in a woman, married, white, of Russian birth, aged 37 years. She was admitted to the hospital complaining of fever, cough, and pain in the right chest. Physical examination showed signs of an active tuberculosis, and this diagnosis was confirmed by the finding of tubercle bacilli in the sputum. Two days after admission, fifteen ounces of fluid were withdrawn from the right chest; and two days later she complained of sudden violent pain over the splenic region. Accompanying this pain there was a moderate degree of shock; five days later this was repeated. She died suddenly on her fourteenth day in the hospital with the signs of a pulmonary embolism.

With the exception of the brain and spinal cord the autopsy

was complete. Briefly stated, the anatomical findings were as follows: *Chest:* double-sided pleurisy with effusion; miliary tuberculosis of both lungs; embolism of the right pulmonary artery. No tumor in the lungs. Heart, normal. Breasts, normal. *Abdomen:* kidneys, both sides, small old infarcts; spleen, recent infarct; uterus and appendages, normal; entire gastrointestinal tract, normal from mouth to anus. The liver was the

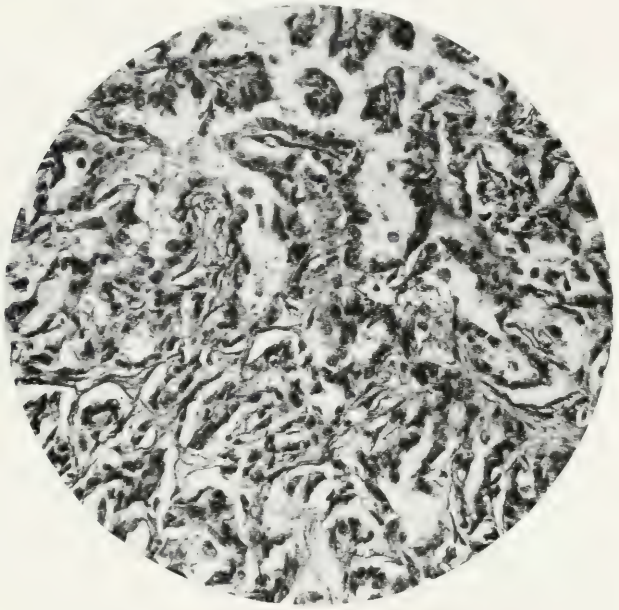

FIG. 1. Carcinoma of the liver, gall-duct type.

chief object of pathological interest. In the right lobe of this organ there was a mass, measuring three inches in diameter, which was visible through the capsule, though it caused no protrusion above the surface of the organ. Section through the liver showed that this mass, white in color, was approximately circular in shape; projecting from it were several finger-like processes. In other areas of the liver, there were scattered small white masses measuring from a quarter to one inch in diameter. The gall-bladder was normal.

Histologically, the main tumor consisted of small epithelial cells of the low columnar type arranged either in alveoli or as papillæ. The individual cells showed a vacuolated protoplasm with a usually central deeply staining nucleus. Mitotic figures were frequently encountered. Sections through the distal portions of the finger-like masses (Fig. 1) showed that they were growing along the bile ducts as emboli. The other areas in the liver showed the same general structure. There were microscopical metastases in the blood-vessels of the lung, and in the retrosternal lymph-nodes.

The interesting features of the case were the coexistence of tuberculosis of the lung and primary carcinoma of the liver. Death occurred from an embolus which was probably derived from the hepatic tumor. The absence of a neoplasm in any organ except the liver, and the presence of microscopic metastases in the lungs and retrosternal lymph-nodes make the diagnosis of a primary hepatic tumor positive. The liver tumor was not suspected during life.

The second case occurred in a man, single, of Irish birth, aged 42 years. Six weeks before admission to the hospital he had had fever, and pain in the right hypochondrium. His physician stated that on the fifth day of his illness, which had a sudden onset, his liver became palpable and had rapidly increased in size, until on admission it reached to the umbilicus. There was no jaundice. On admission there was a leucocytosis of 19,000 with a polynuclear increase to 82 per cent. On palpation the liver was hard and very distinctly nodular. There was fluid in the abdomen. The clinical diagnosis was hepatic cirrhosis, hepatitis of suppurative type, or tumor of the liver. On the third day of his hospital stay the patient became comatose and died.

The autopsy was complete, including the brain and spinal cord. The anatomical conditions present were as follows: Brain and spinal cord normal; lungs normal except for a terminal edema; heart, normal; gastrointestinal tract from mouth to anus normal; prostate, kidneys, and adrenals normal; bladder

normal; spleen, congested and swollen. There were 32 ounces of free fluid in the peritoneal cavity. The chief point of interest was the liver. This was approximately three times its normal size, and was lobulated to a greater degree than is usually seen in the so-called hobnail cirrhosis. The organ was a faint greenish

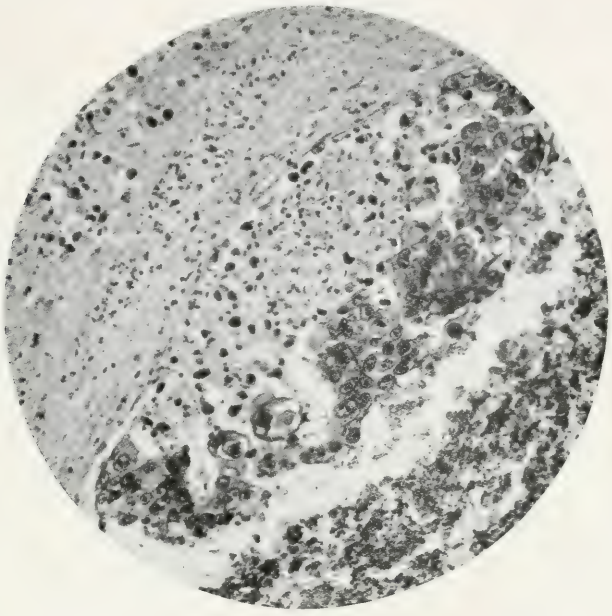

FIG. 2. Tumor embolus of liver cell carcinoma in blood-vessel.

white in color, and section through it showed alternately dense and semi-fluctuating areas. The gall-bladder was normal. Firmly attached to the posterior surface of the organ was a dense mass of retroperitoneal nodes, measuring about six inches in diameter.

Histologically, the liver consisted chiefly of tumor, normal liver tissue being extremely scanty. The individual tumor cells were large and polyhedral in shape, taking a basophile stain and having large nuclei. Mitotic figures were not frequently seen (Figs. 2 and 3). In certain areas tumor cells and normal liver cells apparently merged one into the other; in other areas they

were sharply demarcated. Extensive areas of necrosis were common, the alternating areas of necrosis and healthy tumor tissue giving rise to the hobnailed appearance. Tumor emboli in the blood-vessels were not infrequent. Microscopical metasta-

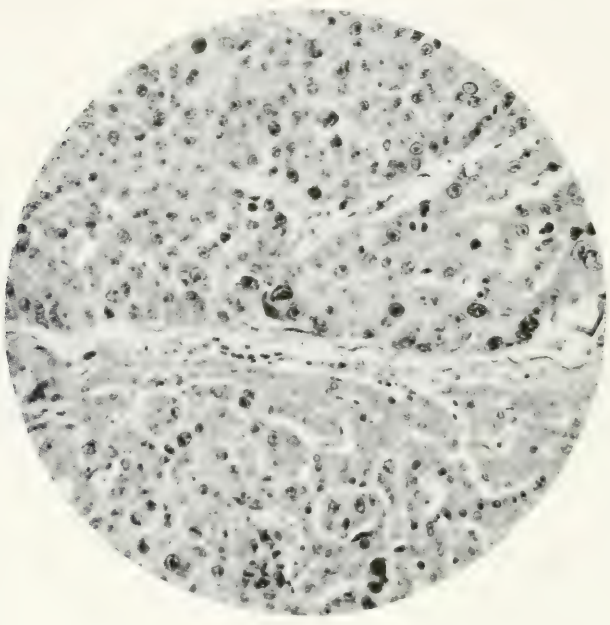

FIG. 3. Hepatoma and normal liver cells adjoining, separated by connective tissue strand. Hepatoma is the larger portion.

ses were demonstrable in the lung, retroperitoneal lymph-nodes, and kidney.

In the process of preparing the tissue for section it was noted that, after transferring the material from formalin to 95 per cent. alcohol, an intense green color developed. This pigment disappeared when the tissue was placed in xylol, and was not given up in any of the reagents used in fixation and dehydration.

An interesting feature of the case, aside from the comparative rareness of the neoplasm, was the very rapid clinical course of seven weeks from the onset of symptoms to death.

Discussion:

Dr. Ewing: Dr. Rohdenburg's paper illustrates many interesting features of primary tumors of the liver. The two cases show the origin of a specific structure, in one case from the bile-duct epithelium, and in the other from the liver cells. The demonstration of the origin of the second tumor from the liver cells seems most satisfactory. The short course of the second case is also characteristic, since most of these cases are discovered only a short time before death, which may occur quite suddenly. It would be interesting to compare closely the clinical courses of the two types of tumors. The presence of metastases in the lymph-nodes is very unusual and possibly unique. I believe that primary hepatoma has not previously been observed to produce genuine progressive metastases. Although there is abundant literature on this subject and several recent studies, it still seems desirable to place such cases on record. This is especially evident since, even recently, prominent Continental writers have interpreted atypical forms of these tumors as angiosarcoma, angioendothelioma, and chorioma.

Dr. Moschcowitz: In a series of six or eight cases which I studied many years ago, much that Dr. Ewing has said was confirmed. In the first place, it was found that metastases were extremely rare; on the other hand, there were often seen finger-like thrombotic masses of tumor projecting into the hepatic veins. Portions of these are frequently carried off and cause emboli in the pulmonary veins, as happened in one of Dr. Rohdenburg's cases. I would like to ask Dr. Rohdenburg whether cancer cells were found in the lung thrombus in his case. Another point is the frequent relationship of cirrhosis of the liver and primary carcinoma. My own studies have led me to believe that liver-cell carcinomata are nearly always associated with cirrhosis, while the gall-duct carcinomata are not and usually exist more in the form of discrete tumors. Indeed, there seems to be a rather close relationship between multiple liver-cell adenomata and primary carcinomata. On gross examination, they often resemble each other very closely, and only a microscopical examination can differentiate them. It often happens that some of the liver nodules show pure adenoma and others carcinoma. Many years ago, a pupil of Ribbert's devised a stain which he believed would distinguish adenoma from carcinoma of the liver, but in my hands this has proved unsuccessful.

Dr. Rohdenburg: In the clinical course, the first case was recognized as tuberculosis, and the confusing point was the very sudden death. The liver tumor was not suspected, and was found only at autopsy. The tumor of the gall-duct type presented no evidence of cirrhosis. The hepatoma was very necrotic, so that one could not say definitely that cirrhosis was present. The gall-bladder tumor presented definite metastases. Many of the metastases of the hepatoma may well have been tumor emboli, and since Dr. Ewing has spoken of it, I recall that many were in recessive phases with degeneration. However, the extension of the hepatoma into the peritoneal glands was distinctly metastatic.

MYOCARDITIS IN CASES OF ENDOCARDITIS

ALFRED G. LANGMANN, M.D.

Out of a total of about 400 autopsies in the Presbyterian Hospital series, the following fifty-four cases, showing definite heart lesions, were selected for study. The listing of these cases in various groups was, in a number of instances, a purely arbitrary matter, and so it happens that the same case will appear in one group because of its chronic lesions and in another group because of its acute lesions. In arriving at this classification the history of past and present illnesses, the gross findings in the heart at autopsy, and the results of bacteriological and clinical tests both during life and after death were taken as a basis.

Accordingly the cases group themselves as follows:

I. Acute rheumatic endocarditis (with or without chronic lesions)	10
II. Acute *Streptococcus viridans* endocarditis (with or without chronic lesions)	5
III. Acute pneumococcic endocarditis (with or without chronic lesions)	5
IV. Acute staphylococcic endocarditis (with or without chronic lesions)	2
V. Chronic rheumatic endocarditis	18
VI. Chronic non-rheumatic endocarditis	11
VII. Syphilitic endo- and myocarditis	6

All these cases were studied with especial reference to the myocardial changes in an endeavor to substantiate the above classification by more or less distinctive findings in the myocardium.

I. *Acute Rheumatic Cases:* In all of the ten cases Aschoff bodies were found in the myocardium. In seven of these, they were numerous, and in one, very numerous. Two of the cases showed only one Aschoff body respectively in several slides; but in both cases the cells composing the nodule and also the grouping were quite typical. In fact, in all but one case (8348) these nodules were found to be quite typical. As a rule, they were found in the midst of muscle tissue, surrounded by a slight or moderate amount of fibrous tissue, or lying adjacent to a blood-

vessel, in certain places the cells appearing strung out alongside the blood-vessel wall. The cells composing these nodules varied somewhat in character, some of them having a fairly deep bluish purple staining cytoplasm with irregular but more or less definite cell outline, others staining a faint purple and showing an extremely hazy and vague cell boundary. The character of the nucleus, also, varied to some extent, some of the nuclei taking on a fairly dark, more or less homogeneous stain, others, somewhat larger in size, appeared as dark circles or ovals inclosing a light field in the center of which the chromatin was gathered in a round or, more often, in an elongated mass.

In practically all the cases there was but slight fibrosis associated with the nodules and in only four cases were definite myocardial scars noted. In two of the cases (8238 and 8457) typical Aschoff bodies were found situated directly in the thickened endocardium, one such nodule causing a distinct bulging of the endocardium. In all of the nodules found, it was noted that the muscle fibers in the immediate vicinity, *i. e.*, those abutting on the tissue in which the nodule was situated, appeared healthy and stained deeply with eosin. There was no gradual transition between these apparently healthy fibers and the Aschoff cells, as one might expect if these latter were derived from degenerating muscle fibers. Furthermore, these cells were in closer approximation to the vessel wall than is the case with normal muscle fibers. In the majority of the cases, the cells resembled most closely the endothelial or mononuclear wandering cells, but the characteristic grouping of the chromatin in the center of the vesicular nucleus was in many instances very striking. In a number of cases, multinucleated cells were seen associated with the mononuclear variety in these nodules. The nuclei of these cells were invariably of the very intense and more or less homogeneous staining variety. In all of the cases the associated round-cell infiltration was very slight.

It should be noted that in three of the cases *Streptococcus viridans* was recovered at autopsy. In one case (8256) the diagnosis of pneumococcus bacteremia was made and pneumococcus

was recovered from the blood during life and from the pericardium post mortem.

Of the ten cases, six gave a history of rheumatic fever at some time during life, and two of these an additional history of chorea. Case 8507 which gave a past history of 6 attacks of rheumatic fever showed relatively few and small myocardial scars and only one Aschoff body was found in several slides. The one case (8348) in which the Aschoff bodies were considered not typical, though suggestive, gave a history of "heart trouble" following scarlet fever, but no rheumatic or choreic history. Only one case showed entire absence of pericarditis; while six had a fairly marked pericardial affection.

(N. B. In those cases in which the Aschoff bodies were quite numerous they occurred not only in the region near the base of the left ventricle but also in other parts of both left and right ventricles and in the interventricular septum.)

II. *Streptococcus Viridans Cases:* These cases show no striking myocardial changes. Several show small focal collections of polynuclear and mononuclear wandering cells; some of these near and others apparently away from blood-vessels. There are, also, small areas of necrotic muscle fibers in which only the sarcolemma and a small pyknotic nucleus remain as evidence of a pre-existing normal muscle fiber. As a rule, there is no cellular infiltration associated with these necrotic areas. In three of the cases small emboli are seen in one vessel of each of the respective cases, and in only one of these cases a fairly marked infiltration of polynuclear cells is seen about the vessel wall.

V. *Chronic Rheumatic Cases:* Of the eighteen cases in this group, all but two gave a more or less definite history of rheumatic fever, and the two exceptions, a history of chorea. Ten of the cases show myocardial scarring of varying degrees, quite pronounced in two instances, and in two cases typical Aschoff bodies were noted. In a third case (8273) numerous small groups of darkly stained mononuclear cells were seen near the endocardium, adjacent to blood-vessels and in the midst of muscle. These cells resemble Aschoff cells but the majority of

them are not characteristic and the grouping is not typical. Pericarditis was present in only eight instances.

In case 8482 in which numerous Aschoff bodies were found there was no evidence of an acute valvular lesion. The patient came to the hospital complaining of abdominal pain and swelling of face, abdomen, and legs of several months' duration, and also of orthopnea and precordial pain. He gave a history of rheumatic arthritis six years before.

The other case (8473) which showed one fairly typical Aschoff body, associated with which are numerous lymphocytes and one eosinophile, gave a history of rheumatic arthritis twenty-nine years before and no subsequent attacks. The myocardium in this case showed several small areas of scarring whereas the preceding case showed none.

III. *Pneumococcus Cases:* Except for one or two collections of lymphocytes the musculature showed nothing especially noteworthy except in one case (8256) which has already been considered in the acute rheumatic group. In this instance, the diagnosis of subacute articular rheumatism was made clinically, although the cause of death was bronchopneumonia and pneumococcus bacteremia. The myocardium in this case showed numerous typical Aschoff bodies. Moreover, this case showed a definite pericarditis, whereas the other four cases showed no such lesion.

IV. *Staphylococcus Cases:* Of the seven cases in this group only three showed definite miliary abscesses scattered throughout the muscle, containing a bluish mass of bacteria at their center. No scars were seen except in one case. The other cases showed no inflammatory changes and no connective tissue increase. (N. B. In each of these cases a staphylococcus bacteremia was found and all but one showed definite lesions of the heart valves.)

VI. *Chronic Non-Rheumatic Cases:* None of the eleven cases gave any history of rheumatic fever or chorea and only four of tonsillitis or sore throat. Three of these four cases showed definite myocardial scars. Another case (8544), also, showed

marked scarring, but in this case there was an associated general arteriosclerosis.

VII. *Syphilitic Cases:* Of the five cases, three showed a cellular infiltration consisting chiefly of lymphocytes and some polynuclear leucocytes. This infiltration was found either about the vessels or in the regions of degenerating muscle fibers which in several sections were quite prominent. One case (8439) showed large mononuclear cells (? wandering cells) scattered here and there between muscle fibers and frequently arranged in cords and even double strands. It is difficult to determine whether these cells were inclosed within capillaries or whether they were simply lining the space between separated muscle fibers. Many of the muscle fibers showed no striations whatsoever, and some of the other cases showed marked changes in their staining reaction. In three instances, marked fibrous scarring was noticed.

CONCLUSIONS

1. All cases of *acute* rheumatic endocarditis studied, ten in number, showed typical Aschoff bodies in the myocardium. Aschoff bodies were found also in two out of eighteen cases of so-called *chronic* rheumatic endocarditis; that is, cases of chronic cardiac valvular disease with a definite history of rheumatic fever or chorea. They could not be demonstrated in any other type of endocarditis.

2. The cases of chronic rheumatic endocarditis did not show myocardial changes which would differentiate them from the non-rheumatic group, with the exception of two in which Aschoff bodies were found. Myocardial scars were present in about fifty per cent. of all cases of chronic endocarditis, and were as common in the non-rheumatic as in the rheumatic group.

3. In three out of seven cases of acute staphylococcus endocarditis, miliary abscesses and bacterial emboli were demonstrated in the myocardium. None of the remaining types of endocarditis studied showed any characteristic myocardial lesion.

Acute Rheumatic Cases

Autopsy No.	Age	Sex	Rheum. Fever	Chorea	Tonsil-litis	Peri-carditis	Valve Lesions	Scars	Aschoff Bodies	
8238	12	F	−	−	+	++	+	+	++	
8348	21	F	−	−	−	++	+	−	+	
8380	17	F	++	−	−	+++	+	−	++	
8444	9	M	−	−	−?	++	+	−	++	
8456	12	F	+	−	−	+	+	+	+++	
8457	4	F	+	−	−	+sl.	+	−	++	
8507	23	F	++	+	−	−	+	+	+	
8568	18	F	+	+	−	++	+	+	++	
8588	11	F	−	−	+	++	+	−	++	
8256	23	F	+	−	+	+	+	−	++	Also listed in Pneumococcus Group.

Streptococcus viridans Cases

Autopsy No.	Age	Sex	Rheum. Fever	Chorea	Peri-carditis	Valve Lesions	Scars	Aschoff Bodies	Emboli	
8275	22	F	−	−	+	+	−	−	+	
8442	38	M	+?	−	++	+	+	−	+	
8518	10	F	−	+	+	+	+	−	+	
8562	36	F	+	−	+	+	−	−	−	Also listed in Chr. Rheum. Group.
8641	24	M	+?	−	−	+	+	−	−	

Pneumococcus Cases

Autopsy No.	Age	Sex	Rheum. Fever	Tonsil-litis	Peri-carditis	Valve Lesions	Scars	Aschoff Bodies	Ab-scesses	
8343	26	F	+	+	−	+	−	−	−	Also listed in Chr. Rheum. Group.
8256	23	F	+	+	+	+	−	+	−	Also listed in Ac. Rheum. Group.
8425	21	F	−	−	−	+	+	−	−	
8536	55	M	−	−	−	+	−	−	−	
8570	39	F	−	+	+sl.	+	−	−	−	

Staphylococcus Cases

Autopsy No.	Age	Sex	Rheum. Fever	Chorea	Tonsil-litis	Pericar-ditis	Valve Lesions	Scars	Aschoff Bodies	Ab-scesses
8494	32	F	−	−	+	−	+	−	−	++
8502	25	F	−	−	−	−	+	−	−	−
8295	23	F	−	−	−	+sl.	+	−	−	−
8270	46	M	−	−	−	−	+	−	−	−
8386	30	M	−	−	−	−	+sl.	+	−	+
8387	19	F	+?	−	+	−	+	−	−	+
8393	20	M	−	−	−	−	−	−	−	−

Chronic Rheumatic Cases

Autopsy No.	Age	Sex	Rheum. Fever	Chorea	Tonsil-litis	Pericar-ditis	Valve Lesions	Scars	Aschoff Bodies	
8254	24	M	+	−	+?	−	+	+	−	
8255	34	F	+	−	−	++	+	+	−	
8273	39	M	+	−	−	−	+	+	+?	
8300	17	M	−	+	−	++	+	−	−	
8312	61	M	+	−	−	+	+	+	−	
8326	28	F	+	−	−	−	+	−	−	
8332	62	M	+	−	−	+	+	++	−	
8415	23	M	+	−	−	+sl.	+	++	−	
8419	20	F	−	+	−	−	+	−	−	
8441	51	F	+	−	−	−	+	+	−	
8473	66	M	+	−	−	−	+	+	+	
8482	13	M	+	−	−	+	+	−	++	
8487	48	M	+	−	−	−	+	−	−	
8503	65	M	+	−	−	+	+	+	−	
8562	36	F	+	−	+	+	+	−	−	Also listed under Strep. virid. Gp.
8343	26	F	+	−	+	−	+	−	−	Also listed in Pneumoc. Gp.
8510	37	M	+	−	−	−	+	+	−	
8210	35	M	+	−	−	−	+	−	−	

Chronic Non-Rheumatic Cases

Autopsy No.	Age	Sex	Rheum. Fever	Chorea	Tonsil-litis	Pericar-ditis	Valve Lesions	Scars	Aschoff Bodies	
8240	10	M	−	−	−	−	+	−	−	
8404	65	F	−	−	−	−	+	+	−	
8407	52	F	−	−	−	−	+	−	−	
8417	45	M	−	−	+	−	+	++	−	
8431	54	M	−	−	−	+sl.	+	−	−	
8449	43	F	−	−	−	−	+	−	−	
8466	5	M	−	−	+	−	+	−	−	
8497	45	F	−	−	−	−	+	−	−	
8529	35	M	−	−	++	+	+	+	−	
8539	51	F	−	−	++	−	+	+	−	
8544	59	F	−	−	−	−	+	++	−	

Syphilitic Cases

Autopsy No.	Age	Sex	Rheum. Fever	Chorea	Pericar-ditis	Valve Lesions	Scars	Aschoff Bodies	Wasser. React.
8439	42	M	+?	−	−	+	++	−	++++
8453	43	M	−	−	+sl.	+	−	−	++++
8476	60	M	−	−	+sl.	+	−	−	Not obtained
8534	33	M	−	−	−	+	++	−	++++
8549	48	M	−	−	−	+	+	−	+
8599	46	M	−	−	+sl.	+	++	−	++++

CULTIVATION OF A PROTOZOAN BY ORDINARY BACTERIOLOGICAL METHODS. COLPODA CUCULLUS.

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The ultimate purpose of the work which is here reported was the study of antibody formation against protein derived from protozoa. It was necessary to obtain a cultivable protozoan which could be grown in bulk sufficient to permit antigen in quantity. Experiments were first made with an ameba. Dr. Anna Williams supplied several cultures of a dysentery ameba growing on pieces of guinea-pig brain, but these organisms were very small and it was impossible to get them in sufficient quantity for our particular purposes. Infusions were started in the hope of finding *Amœba proteus,* which is large and viable on artificial media.

After two or three days, many protozoa appeared in a hay infusion, among them a ciliate which developed in great quantities. Several of these were fished from drops of the infusion by means of a capillary pipette and transferred to tubes containing 10 c.c. of sterile, slightly acid hay infusion. The organisms grew well, and in a couple of days a cloudy white substance could be seen throughout the media; by the end of two weeks this had precipitated, leaving a perfectly clear supernatant fluid. Agar plates, inoculated with the sediment, showed that several varieties of bacteria—*Staphylococcus aureus, Bacillus pyocyaneus,* hay bacillus, a spirillum, and a motile spore-bearing organism—were present with the protozoa. This protozoan, found in the sediment, was subsequently identified as a *Colpoda cucullus.* It may be described as follows: When first seen it had a definite, rigid outline, much like a cucumber-pickle; was uniformly covered with lines of cilia which gave a notched appearance to the anterior end; mouth in oral groove about one third from the

anterior end; large nucleus slightly to one side of the center; and one large characteristic contractile vacuole at the posterior end. By the end of the third week all the protozoa disappeared from the supernatant fluid and a gradual change was observed in those in the precipitate. The organism no longer swam rapidly about, and soon lost its definite shape; becoming plastic and polymorphic it oozed through the gelatinous matter it had secreted, more like an ameba in movement, though the beating of the cilia could still be seen. Later it moved only in one spot, circling slowly about and finally it became quiescent, retaining a rounded outline and still showing the nucleus and contractile vacuole; at the end of a few hours a definite membrane seemed to appear around the organism and by the end of twenty-four hours had become a heavy yellowish cyst through which no morphological structures could be seen. Several days after transplanting to fresh media there appeared clusters of tiny, colorless cells, unobserved in any of the previous cultures. Upon examination the following day organisms appeared which possessed pseudopodia by means of which they moved sluggishly through the medium. At first, we were in doubt whether these were the same organisms. After forty-eight hours, free-swimming ciliates had appeared, in form somewhat like the large *Colpoda*. It is, of course, difficult to say whether these were the known stages of development of one and the same organism.

To show that this was a stage in the life history of *Colpoda*, rather than some other protozoan, cysts of the *Colpoda* were carefully isolated and washed in sterile salt solution; then tubes of sterile hay infusion were inoculated with these washed cysts, and development was watched. The same cycle was apparently repeated, terminating in full-grown *Colpoda*. It seems at least probable, therefore, that these various stages represent the life history of one organism.

It was observed that any change in temperature hastened the encystment, and that the organism remained in this state until transplanted; this may be attributed to several causes either the food supply was exhausted or was not of the right character, or

there had been some chemical change in the media which made this protective measure necessary. Bacteriological examination showed that there were bacteria in the sediment and chemical examination showed that the infusion, slightly acid when inoculated, had become decidedly alkaline. Pure cultures of the different types of bacteria, therefore, were started. From these agar slants were inoculated, and carefully washed cysts were planted in the condensation fluid. After twenty-four hours, small clusters of colorless protozoa could be seen in the condensation fluid of the tubes of *Staphylococcus pyogenes aureus* and of *Bacillus pyocyaneus*. The protozoa developed to the small free-swimming stage under these conditions, but even frequent transplantation to fresh agar slants has not succeeded in producing the adult forms. Large free-swimming forms were also washed in sterile water and planted in the same way in the condensation fluid of agar slants and in the extract broth. They did not increase in the condensation fluid and soon died out, due, probably, to the more rapid growth of bacteria. In the extract broth, both flourished for a time; then the protozoa became filled with food particles which collected in a dark mass at the posterior end; this showed that they were not excreting properly and needed a change of media. Those transplanted to normal salt solution promptly died, while those transplanted to tubes of hay infusion + 3 c.c. of salt solution seemed benefited and cleared up gradually, after several transplantations. Others put into fresh tubes of hay extract broth grew well for a time, but the bacteria increased so rapidly that the media became cloudy; a heavy sediment, unlike the cloudy white substance found in the first tubes, and a thick pellicle appeared.

The *Colpodæ* were found near the top of the fluid and also in the pellicle. Tubes of encysted organisms were found to have become decidedly alkaline, some of them were, therefore, neutralized with $N/20$HCl, then made slightly acid; the following day the cysts were found to have ruptured and the tiny clusters were observed lying close beside the broken cyst. They did not mature in these tubes, but upon transplantation developed rapidly.

Plates made of infusion agar were inoculated from tubes of both free-swimming and encysted *Colpodæ* and in both sets bacteria developed, but no *Colpodæ* were found on the solid media. On gelatine and Hiss plate medium, growth was fairly good, at first, but was probably inhibited, finally, by the more rapid growth of bacteria. Fresh pure cultures of the different bacteria were begun on infusion agar slants, and it was found possible to obtain by this process cultures of *Colpodæ* and a single strain of bacteria—either *Bacillus pyocyaneus* or *Staphylococcus pyogenes aureus*. From these, tubes of hay infusion were inoculated and the protozoa grew well, but frequent transplantation was required to produce this result. New cultures, made at least once each week, will keep the *Colpodæ* in a free-swimming condition for months. Left in the same media for a longer period than a week they soon begin to encyst, the cloudy white precipitate becomes darker and flaky, until, by the time all the organisms have encysted, it is a deep brown in color and has lost the gelatinous consistency which is characteristic while there are yet free-swimming forms in the media.

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Dr. Eli Moschcowitz, Acting President.

A POLYPOID MYXOMA OF THE HEART

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The specimen which I wish to present is an example of the so-called polypoid myxoma of the heart, a very rare tumor,

though probably the most common one originating in the heart, which, as you know, is rarely the seat of primary new growths. In a series of twenty-eight cardiac neoplasms collected by Berthenson in 1895, myxoma ranked second in frequency; but in a larger series of ninety-one cases, reviewed by Lind in 1909, this tumor stood first, there being eighteen cases of myxoma and thirteen of sarcoma, the second in frequency. Of the total number of myxomata of the heart reported up to the present time, only three have been described in American literature, whereas at least twenty have been recorded in the German journals of the past twenty-five years. This tumor appears to have excited special interest among the German pathologists, not so much because of its comparative rarity among tumors in general, as because of the discussion which arose over the question as to whether or not the tumor is a true neoplasm. I shall take up this question after presenting my case.

The patient from whom the specimen was obtained was a woman, aged 53, who was admitted to the Presbyterian Hospital in July, 1917, complaining of shortness of breath and general weakness of four months' duration. Her family history was irrelevant, her past history negative. She had never had an attack of acute rheumatic fever, and there was no evidence in the history of previous circulatory disturbance. Her chief symptoms, weakness and shortness of breath, began soon after a severe cold contracted during March, 1917, four months before death. Three days before admission to the hospital, weakness increased and dyspnea became very distressing. The findings on physical examination upon entrance were those referable to a state of cardiac insufficiency. The heart was definitely enlarged; the area of cardiac dullness extended 13 cm. to the left of the midline in the fifth intercostal space. Heart sounds were loud and ringing; there were no murmurs. On the third day in the hospital, after turning suddenly in bed, the patient stopped breathing; the heart continued beating feebly for a few moments, and then ceased.

The autopsy was performed six hours after death. The

findings, except for those in the heart, were unimportant. There was moderate chronic passive congestion of all the viscera.

FIG. 1. Polypoid myxoma of heart. Photograph of gross specimen. Note size and location of tumor, nodular surface, and deep anterior furrow.

The right side of the heart was found in a state of marked dilatation, while the left ventricle was firmly contracted. On opening the left auricle, its wall was seen to be considerably hypertrophied, measuring 3 mm. or more in thickness. Occupy-

ing practically its entire cavity was a large, glistening, gelatinous tumor mass measuring 5 cm. in width and 6.5 cm. in length (Fig. 1). It was attached by a narrow stalk, 1 cm. in diameter, to the interauricular septum just anterior to the fossa ovalis. The most striking features of the tumor, which immediately impressed those present at the autopsy, were its remarkable translucency and the glossy character of its surface, different from the dull, lusterless appearance of the ordinary cardiac thrombus. Covering the entire surface of the tumor were numerous, slender, villous-like projections, characteristic of papillomatous tumors. Along one of the lateral surfaces there was a narrow, but fairly deep furrow, which, judging from its general direction, might have served during life to conduct the blood from the upper and back part of the left atrium, downwards and forwards to the mitral orifice.

The histological picture of the tumor was quite interesting. The free margin of the section was very irregular, corresponding to the villous-like projections on the gross specimen. The surface was covered by a single layer of cells with deep blue-staining cytoplasm, and oval vesicular nuclei (Fig. 2). The cells were more flattened at the summit of the projections than in the depths of the intervening depressions. Many of the cells possessed branching protoplasmic processes which extended into the substance of the tumor. The general appearance of the cells suggested that they were not endothelial cells of the ordinary type, but connective tissue cells in the process of adaptation to pressure conditions within the atrium. The histological structure of the tumor proper varied in different areas. Immediately beneath the surface, there was seen a loose blue-staining matrix in which were scattered many branching connective-tissue cells. The picture was practically identical with that of Wharton's jelly of the umbilical cord. Towards the deeper parts of the tumor, the matrix stained less deeply than in the superficial areas, and in addition there appeared large, irregular spaces lined by cells similar to those covering the surface. Some of these spaces contained well-preserved red blood cells. Here and there, also,

were small discrete masses of pink-staining fibrin, which showed no tendency to organization and had the appearance of being freshly formed. They did not seem to bear any recognizable relation to the other structural elements of the tumor. At the stalk there was a considerable amount of yellow granular pigment (hemosiderin) not found in other parts of the tumor; most of it had been taken up by large phagocytes.

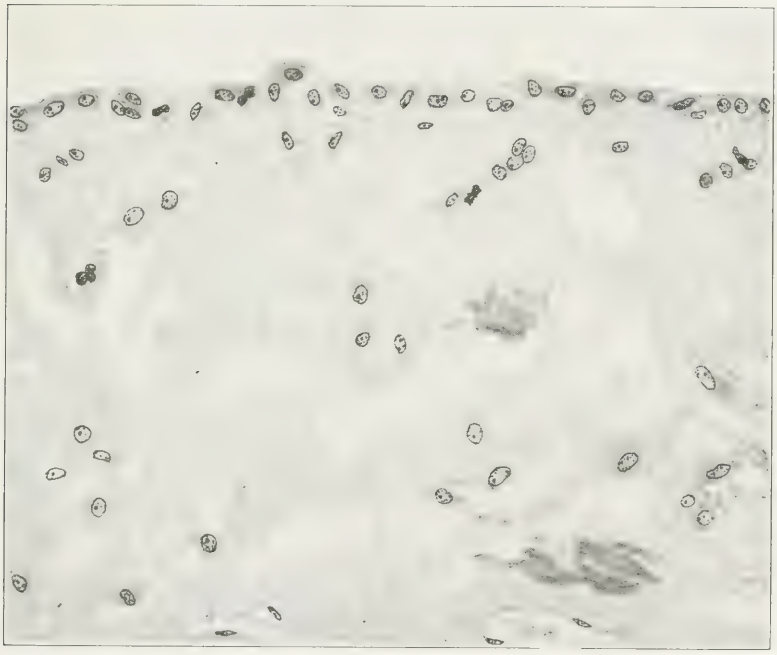

FIG. 2. Polypoid myxoma of heart. Drawing showing layer of endothelial cells covering surface of tumor, and loose, relatively non-cellular myxomatous tissue beneath.

The question now arises as to the interpretation of this pathological specimen. In practically all its characteristics it is identical with the majority of myxomata of the heart reported in the German literature. It is needless to point out that the gross appearance of the tumor is entirely different from that of an ordinary cardiac thrombus. Histologically, the presence of

isolated fibrin masses and blood pigment would seem to lend support to the view that the "tumor" is nothing more than an organizing thrombus, but in view of the absence of the other histological characteristics of a thrombus, one is justified, I think, in accepting a different explanation of the origin of the fibrin and pigment.

In the German literature of the past twenty-five years there has been considerable discussion as to the nature of the cardiac polyp; and it may not be amiss at this point to review briefly the main ideas which have been advanced. In his several reviews of the subject, Thorel has maintained that the cardiac polyp, such as I have described, is not a true tumor, but has its origin in a cardiac thrombus. He believes that the connective tissue of the organized thrombus undergoes edematous changes with transudation of fluid into the space between the cells and fibers, and that subsequently chemical changes take place in the edematous fluid, resulting in the production of mucin. This process is termed by Thorel "myxomatous metamorphosis" or "myxomatous degeneration." This change results in a tissue differing greatly in appearance, macroscopically and microscopically, from the ordinary organizing thrombus. Among those opposed to this view is Ribbert, who maintains that the cardiac polyp is a true tumor. He is inclined to doubt the occurrence of "myxomatous metamorphosis"; and believes, on the contrary, that myxomatous tissue is an independent variety of connective tissue, and that tumors composed either wholly or in part of myxomatous tissue should not be looked upon as the result of secondary change in some other tissue but as the product of true tumor growth. It is obvious that the whole question of the nature of cardiac polyp hinges upon the existence or nonexistence of myxomatous degeneration.

Quite a number of other able pathologists, including MacCallum, regard the occurrence of true myxomatous degeneration as very doubtful. It is their opinion that the burden of proof rests upon those who maintain that the cardiac polyp is simply an organized thrombus with myxomatous degeneration, and that

until such proof is submitted the cardiac polyp should be classified as a true myxoma.

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Discussion:

Dr. Lambert: I was present at the autopsy which Mr. Louria has described and was very much interested in the findings. There is one point upon which I do not think he has laid sufficient emphasis. That is the absence of any cause of circulatory disturbance other than the polypoid tumor of the heart. If, as some writers have maintained, these tumors are nothing more than organized thrombi, one should find in every case a cause for thrombus formation. A second point which might be emphasized is that these polypoid tumors are usually attached to the inner wall of the auricle just anterior to the fossa ovalis, an area, as Ribbert has pointed out, in which an embryonic displacement of tissues favorable to tumor development is likely to occur.

Dr. Moschcowitz: I should like to ask Mr. Louria if the patient had delirium cordis.

Mr. Louria: I did not see the patient clinically.

ATYPICAL HORSESHOE KIDNEY

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Anomalies of the kidney fall into several well-recognized groups, of which the more important are unilateral congenital atrophy, fused kidney, solitary kidney, and lobulated kidney. While this specimen does not correspond exactly with any of the commoner types described, it may be regarded as an atypical horseshoe kidney.

In the clinical history of the case there was nothing to indi-

cate the existence of renal anomaly. The patient, a negro man of 52 years, had been admitted to the hospital four times during the last two years of his life, complaining generally of dyspnea, edema of extremities, and lassitude. Physical examination

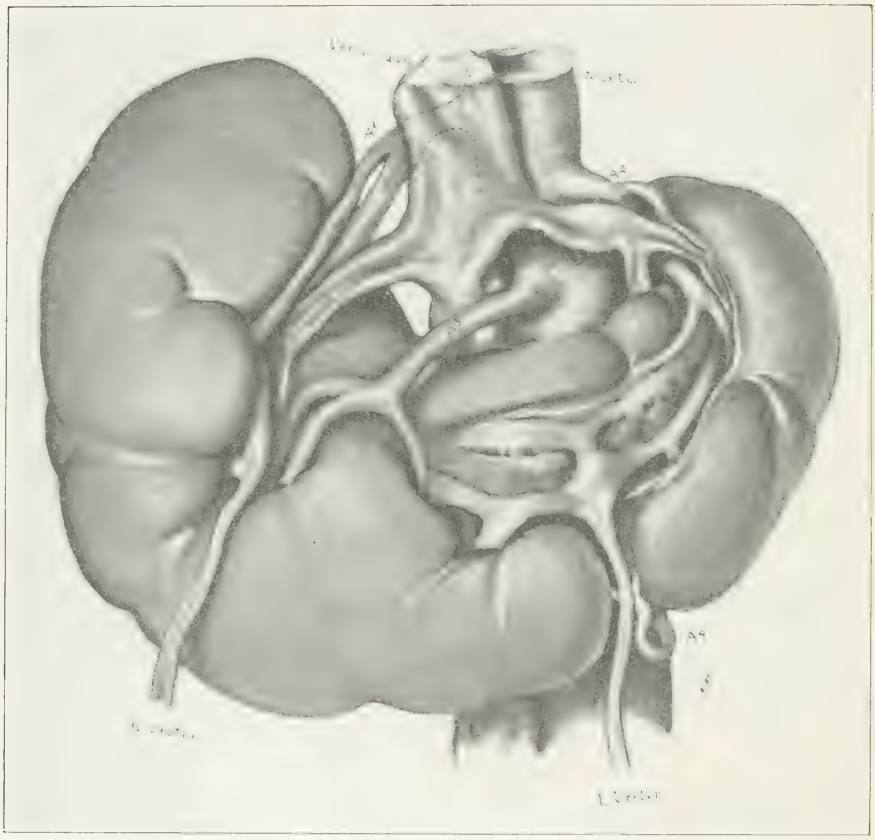

Fig. 1. Atypical horseshoe kidney. A^1, A^2, A^3, A^4, renal arteries.

showed evidences of arteriosclerosis and chronic myocarditis with cardiac insufficiency and glycosuria. The circulatory condition generally improved under treatment, and the sugar disappeared from the urine with a proper diet. During his last stay in the hospital the sugar persisted in the urine, and gangrene of

the right foot developed, followed by general anasarca, coma, and death. The specific gravity of the urine was 1.030 to 1.040, and there was a trace of albumin, but no casts or cells. The systolic blood pressure was 166, the diastolic 70.

At autopsy there was found, in addition to the renal anomaly, a generalized arteriosclerosis, involving especially the aorta, and the coronary and cerebral vessels, chronic passive congestion of the organs, and a chronic intralobular pancreatitis. Only the kidneys, however, need be described. When the intestines were removed, a big mass of kidney tissue was seen to the right of the vertebral column, with only a small mass in the region of the left kidney. Kidneys, ureters, and bladder were then removed *en masse* and carefully dissected. The right kidney was found to be fully twice as large as the left, and there was a broad mass of kidney substance extending between the two from the hilus to the lower poles. The upper part of this uniting band projected further forward than the lower, as is shown in the accompanying illustration (Fig. 1). Posteriorly, there was a uniform sheet of kidney tissue connecting the two organs. The renal vessels and ureters showed also certain abnormalities in their origin and branching. There were four arteries. The first (A^1) left the aorta rather high up on the right side and divided immediately into two branches, which supplied the upper portion of the right kidney. The second (A^2) came off on the left side about opposite the left renal vein and gave branches to the small left kidney and to a part of the medial sheet of kidney tissue. The third (A^3) was an anterior branch of the aorta, starting just below the renal veins and supplying the lower portion of the right kidney. The fourth (A^4) was given off at a point about opposite the lower border of the kidney, that is, only 4 cm. above the bifurcation. It supplied the lower portion of the renal isthmus. The veins showed no definite abnormalities.

The two ureters pursued a normal course after leaving the kidney, but were anomalous in their beginnings. The right drained only the upper portion of the right kidney, while the left was formed by branches from the left kidney, the lower portion

of the right, and the medial communicating mass of kidney tissue.

It is evident from this brief description that this specimen is not a typical horseshoe kidney, although it undoubtedly belongs to that general group. Eisendrath divides fused kidneys into three classes: (1) Typical horseshoe kidney with fusion confined to the lower poles; (2) intermediate form of solitary kidney; (3) disc-like kidney, where both organs are fused into one disc-shaped mass. This specimen falls into the second class of intermediate solitary kidney. A similar case has been described by Küster.

Horseshoe kidneys are not rare. They constitute by far the most common form of renal malformation. Keyes, in 11,000 autopsies, found 53 malformed kidneys, and 16 of these were of the horseshoe type. The origin of the fused kidney is fairly well understood. The two mesothelial bodies, which are the "anlagen" of the renal parenchyma, unite with the outgrowths from the Wolffian ducts, which form the collecting radicals of the renal system. These primitive structures gradually ascend from the pelvis to their final resting place. It is before this ascent or during it that the lower poles may approximate and become fused. The amount of kidney tissue involved in this disturbance determines the character and conformation of the anomaly. According to Charles Mayo, anomalies of the blood vessels are due to the persistence of some of the vessels of the plexus which surrounds the renal "anlage" during embryonic life.

The clinical significance of fused kidneys has received a good deal of attention. Rovsing, after studying four cases, constructed a clinical picture of horseshoe kidney, the chief feature of which is pain across the abdomen induced by exercise, exaggerated when the patient bends forward, and relieved by lying down. He believes that such masses of kidney tissue can be palpated in front of the great vessels in individuals with lax abdominal walls. Eisendrath has called attention to the relative frequency of hydronephrosis and pyonephrosis in horseshoe kid-

neys, and cites two cases in which a diagnosis of horseshoe kidney was made before operation.

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Discussion:

DR. A. L. LOURIA: The clinical picture that Dr. Garlock suggests interests me because of a case I saw two years ago. The patient was a girl, twenty years old, who complained of pain just below the umbilicus. She was well nourished and did not look ill. Physical examination revealed nothing beyond a mass just above the level of the sacrum, about two inches broad, tender, and definitely overlying the aorta and pressing upon it, as evidenced by a bruit and a transmitted, non-expansile pulsation. The diagnosis of horseshoe kidney was made, and this was subsequently confirmed at operation at the Jewish Hospital.

DR. MOSCHCOWITZ: It seems to me it would be very interesting to determine the relation of the pelves by *x*-raying the kidney after the injection of collargol.

DR. LEON LOURIA: In the case just mentioned, which was sent to the Jewish Hospital of Brooklyn, the diagnosis was made before operation. We injected thorium, which affirmed the diagnosis and gave a beautiful picture of the relation of the pelves of the kidneys. The patient was operated upon successfully and relieved of her pain. It is interesting to note further that, while her symptoms were temporarily alleviated, she returned to the hospital some time later with a recurrence of the abdominal pain. On examination a mass, similar to that before operation, was readily palpated. It appears that the severed poles had reunited. Unfortunately her symptoms subsided during her stay in the hospital, and she refused a second operation, so that we were unable to substantiate our assumption. It would be interesting to learn if surgery can completely relieve such anomalies.

DR. WILLIAM LINDER: My only experience in operating upon a horseshoe kidney is in the case referred to me by Dr. Leon Louria. A previous diagnosis having been made, I made a right rectus incision and entered the peritoneum. After the intestines had been thoroughly packed away, the posterior layer of the peritoneum overlying the kidney was incised and the kidney exposed. The blood supply was studied and the ureters identified; the kidney was then severed and a wedge-shape piece removed from each part in order to reconstruct the lower pole. I was not satisfied until we

could place the left kidney to the left of the spinal column. Subsequent examination of the patient revealed a probable reunion of the severed kidney. I believe that the fibrous capsule which enveloped the entire kidney had probably pulled the poles together. In the next case, I propose making a median incision and going through intraperitoneally in order that I may be able to fix one kidney higher and decapsulate the other, so that a union can not take place. Perhaps we can thus avoid the fusion of the two poles.

MALIGNANT NEUROBLASTOMA

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The term "malignant neuroblastoma" has been applied in the last few years to a tumor occurring generally in infants or young children and arising from the undifferentiated tissue of the sympathetic nervous system. The location of the primary tumor is most often in the adrenals, less commonly in the retroperitoneum or mediastinum; but owing to the wide distribution of sympathetic nervous tissue, and the migratory power of its formative cells, the tumor may, theoretically, at least, have its origin almost anywhere in the body.

The true nature of this tumor was not generally recognized until a few years ago. In the eighties, nineties, and even as late as 1910, there were reported cases of lymphosarcoma, round-cell sarcoma, glioma, and gliosarcoma of the adrenals which, it is evident in the light of recent studies, were examples of neuroblastoma. In 1891, Marchand described a tumor of the adrenal and pointed out the resemblance of the cells to those of embryonic nerve tissue; it was not, however, until 1905 that the histogenesis of this group of tumors was definitely established. In that year Küster, a pupil of Ribbert, reported two tumors of the adrenal in children, which he interpreted as gliomata. Wiesel, who had made a special study of the development of the sympathetic nervous system, and especially of the origin of the adrenal medulla, through the invasion of the "anlage" of the adrenal cortex by formative cells of the sympathetic, called at-

tention to the similarity between the tissues composing Küster's tumors and these undifferentiated formative cells, or "Bildungszellen," pointing out that the morphology of the cells and the character of the fibrils in the neoplastic and embryonic tissues were practically identical. Wiesel's contention was not disputed, but tumors of the adrenal of this type were still reported under various names until 1910, when Wright published a brief but complete review of the question, a review which has secured for this tumor fairly general recognition. He collected from the literature eight cases which, from descriptions and illustrations, could be placed in the neuroblastoma group; and to this number he added four cases of his own. In his paper, Wright set down concisely the distinguishing histological characteristics of the tumor; these may be summarized as follows: The tumors are rich in cells, which may vary much in size and shape. They may be pervaded by connective tissue, the cells of which are arranged in more or less definite alveoli. The essential cells of the tumor are considered to be undifferentiated nerve cells, neurocytes or neuroblasts, for the following reasons: First, the cells, at least in places, are associated with delicate fibrils, often of considerable length, which do not stain like neuroglia, collagen, or fibroglia fibrils by Mallory's methods, and which are like the fibrils occurring in the "anlage" of the sympathetic nervous system; second, cells associated with these fibrils have the same morphology as the cells from which the sympathetic nervous system and the medulla of the adrenal develop, which are regarded by embryologists as arising from migrated primitive nerve cells. They are generally small, with round nuclei rich in chromatin, and have a relatively small or imperceptible amount of cytoplasm. Some of them may be pyriform in shape and their cytoplasm may be prolonged into filamentous processes like the fibrils referred to above; third, the cells and fibrils in places are arranged more or less like the neurocytes and their fibrils in the sympathetic nervous system at certain periods of its development and in the "anlage" of the adrenal. The fibrils may be arranged parallel to one another in bundles which are intimately associated with masses of cells, or they may be seen as a mesh-

work in the center of ball-like aggregations of cells to which the name "rosette" has been applied.

Since Wright's paper was published, at least eleven other cases of malignant neuroblastoma have been reported, and several excellent reviews of the subject of tumors of the sympathetic system have appeared. One of the best of these is by Wahl, in which there is a thorough discussion of all three of the well-recognized tumors originating from sympathetic nervous tissue, namely, *malignant neuroblastoma,* arising from the undifferentiated sympathetic formative cells, *ganglioneuroma,* from differentiated nerve fibers and ganglion cells, and *paraganglioma,* from chromaffin cells. The relations of these three tumors to one another and to the tissues of the sympathetic system out of which they develop are well shown in the following diagrams taken, with some modifications, from Wahl's paper.

CHART I

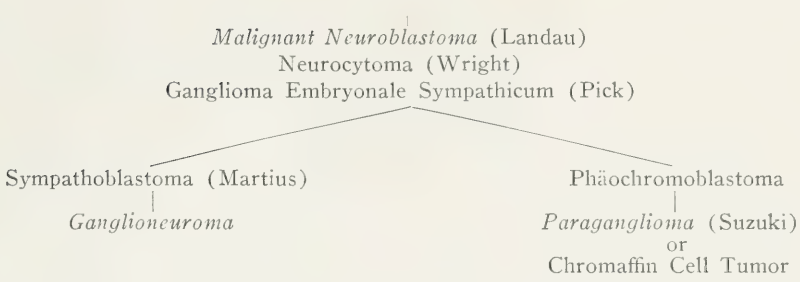
Neuroectoderm of Medullary Tube

Sympathogonia
or
Sympathetic Formative Cells ("Bildungszellen" of Wiesel)

Sympathetic System	Sympathoblasts or Transition Cells	Phäochromoblasts
	Ganglion Cells and Sheath Cells	Phäochromocytes or Chromaffin Cells
		Chromaffin System

In the second diagram, in addition to the three well-recognized tumors emphasized by italics, the names of two other tumors are given, sympathoblastoma or transition-cell tumor, and phäochromoblastoma, a tumor made up of undifferentiated chromaffin cells. Only a single case of sympathoblastoma has been reported (Martius, 1913), and that was not a pure example, showing in places differentiated nerve cells and fibrils. Phäochromoblastoma is only a theoretical possibility, no such tumor having yet been described.

CHART II



Of the three names proposed for the malignant tumor developing out of differentiated formative cells, malignant neuroblastoma, proposed by Landau, is to be preferred. The term "neurocytoma" should not be used for this tumor, since it has been given already by Marchand to a tumor of the cerebrospinal system made up of entirely undifferentiated cells without fibril formation. Pick's term, "ganglioma embryonale sympathicum," is inaccurate, as Wahl has pointed out, because it implies the presence of either differentiated or undifferentiated ganglion cells, whereas the formative cells of the tumor may give rise to either ganglion cells or chromaffin cells.

The writer's interest in tumors of the sympathetic system was aroused by the study of a case of malignant neuroblastoma which came to autopsy recently at the Presbyterian Hospital. The relative infrequency of this tumor is the chief excuse for presenting a report of it to the Society. Additional interest may be given the case, however, by the fact that it does present certain atypical features.

Clinical History.—Girl, aged five years, had diarrhea when eight months old, and measles at three years. Seven months ago she had an abscess of the neck which healed readily after surgical incision.

Present illness began four and a half months before admission to hospital, that is, six months before death, with severe cold, accompanied by cough and fever. After a few days in bed, the patient developed shifting pains in joints, abdominal pain after eating, and attacks of frontal headache every day or

two; she lost weight steadily with progressive weakness and anemia; had been continually in bed. Enlargement of axillary and cervical lymph-nodes was noticed six weeks before coming to the hospital, that is, three months after onset of illness.

Physical examination on admission showed marked enlargement of axillary, cervical, and inguinal lymph-nodes, prominent nodules on right frontal bone, right-sided exophthalmos, distended abdomen, enlargement of heart to left, and anemia.

The red blood cells numbered 2,400,000; the hemoglobin was 41 per cent. There were 6,000 leucocytes, with 72 per cent. of neutrophiles. There were no nucleated red cells. The Wassermann reaction was negative. Examination of the urine was negative (no Bence-Jones protein). An *x*-ray plate showed extensive rarefaction of pelvic bones, lumbar vertebræ, tibia, and skull.

The patient died after six weeks in the hospital. During this time there was irregular remittent fever, generally 100° to 102°, pulse 120 to 140. Weakness increased progressively with enlargement of the lymph-nodes, especially the axillary and cervical. A large abdominal mass could be palpated in the region of the umbilicus, and another about the rectum. Exophthalmos became pronounced in both eyes. A lymph-node was excised, and microscopic examination of this established the diagnosis of malignant neuroblastoma.

The autopsy was performed sixteen hours after death. The body was markedly emaciated and small for the age. Exophthalmos was pronounced in both eyes, most marked in the left. The glands of the neck, axilla, and inguinal region on both sides were greatly enlarged, but fairly discrete; the popliteal glands were palpable; those of the epitrochlear region were not. On the right frontal bone there was a rounded firm prominence, 5 cm. in breadth, over which the skin moved readily. Less porminent nodules could be felt over the entire vertex.

Thorax.—There was a small thymic fat pad, weighing about 15 gm., but little visible thymic tissue. There was a slight excess of fluid in both pleural cavities, with organized adhesions

FIG. 1. Neuroblastoma: Retroperitoneal tumor with involvement of peripancreatic, aortic, and pelvic lymph-nodes. Adrenals, kidneys, ureters, bladder, and rectum, partially surrounded but not invaded by tumor.

about the apex of the left lung. *Heart and lungs* showed nothing pathological except pallor of the cardiac muscle. After their removal, a mass of tumor tissue was seen in the posterior mediastinum, surrounding the descending aorta, and small nodules were firmly attached to the ribs in various places. These nodules extended into the ribs and were obviously metastases in the bone which had broken through the periosteum. The costal pleura over them was smooth.

Abdomen.—The peritoneal cavity contained about 100 c.c. of clear yellow fluid. The liver extended 3 cm. below the costal margin; the kidneys and spleen appeared to be in normal positions. The stomach and pancreas were pushed forward by a large, firm, nodular mass in the retroperitoneum (Fig. 1). The mass had no definite outline, but extended down along the aorta, and was continuous with the enlarged iliac and inguinal nodes. The mesenteric nodes were small, none exceeding 0.5 cm. in diameter.

The *liver* weighed 700 gm. and like all the other organs was pale, almost white when the blood was washed off. The lobules were faintly seen, but were of normal size; there were no tumor nodules.

The *spleen* weighed 75 gm. The Malpighian bodies were distinctly seen as white, opaque dots scattered uniformly through the pulp; there was no gross evidence of tumor.

The left *adrenal* could be easily dissected from the retroperitoneal mass; it was quite thin, but the cortex and medulla were distinctly outlined. The right *adrenal* was partially surrounded by tumor, but not infiltrated by it (Fig. 2). Like the left, it was very thin, as though compressed by the adjacent tumor mass.

The *kidneys, bladder, uterus, adnexa, esophagus, stomach,* and *intestine* showed nothing pathological.

In opening the *skull* the saw line was carried through the prominence on the right forehead. This was found to be tumor, evidently a metastatic nodule in the frontal bone. Similar smaller metastases were present in other portions of the skull. The metastases in the frontal bones had extended through the

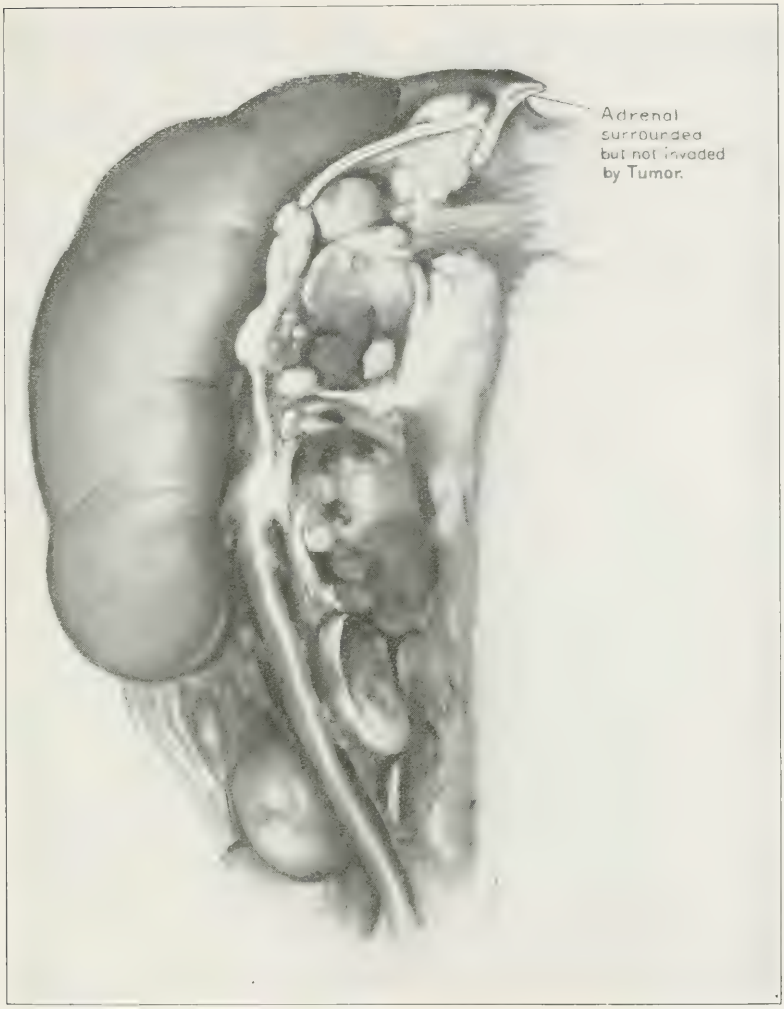


FIG. 2. Right kidney, adrenal, and retroperitoneal tumor. Neighboring lymph-nodes are replaced by tumor, but kidney and adrenal are not invaded.

dura and invaded both frontal lobes. There was a similar invasion of the right parietal lobe. A mass of tumor projected into the cranium from each orbital fossa. Multiple sections through the *brain* showed no metastases except those which had invaded it through the skull.

The size of the main abdominal tumor with its anatomical relations is very well shown in the first cut. The tumor had no limiting capsule and had spread widely through the retroperitoneal tissues. There was, however, no macroscopic infiltration of any of the adjacent viscera—pancreas, spleen, liver, adrenals, or kidneys. A large part of the tumor mass was made up of big, partially fused, retroperitoneal lymph-nodes which were entirely occupied by neoplastic tissue. The iliac and inguinal nodes were similarly involved, but were fairly discrete with little or no evidence of invasion of their capsules. This was true also of the axillary and cervical nodes, some of which measured 5 to 6 cm. in diameter. The tracheal nodes were smaller, but in places their capsules had been invaded with an extension of the tumor to the adjacent muscles. The glands at the hilum of the lung were not involved. There was, however, a continuous stretch of tumor along the entire length of the aorta, in which the capsule of a node could now and then be made out. The psoas muscles were extensively infiltrated. In the deeper portion of the pelvis, around the rectum, there was a big mass of tumor which was continuous with the pelvic bones. It was difficult to make out which way the tumor was growing, whether from the bones into the pelvis or vice versa.

The character of the tumor tissue was very much the same everywhere. It was firm, but not hard, and moderately friable. The color in most places was a uniform gray, and the tissue was semi-translucent. There was only an occasional fibrous septum, and no macroscopic foci of necrosis or softening. There were a few small hemorrhages in the cervical and axillary nodes; and in the retroperitoneal tumor several extensive hemorrhagic areas were present. These were seen also in the metastases in the skull bones.

It is not possible to say with absolute certainty just where the tumor originated, but the greater size of the retroperitoneal growth makes it probable that the tumor began there.

Microscopical Examination.—The tumor showed every-

where the same histological features; and the description of the inguinal node removed during life applies equally well to the tumor found at autopsy:

The growth was composed of cells relatively small in size, with round, chromatin-rich nuclei and scanty cytoplasm. There was a tendency toward an alveolar arrangement of the cells, which was quite pronounced in some sections. In places there were aggregations of cells arranged in circles with a central,

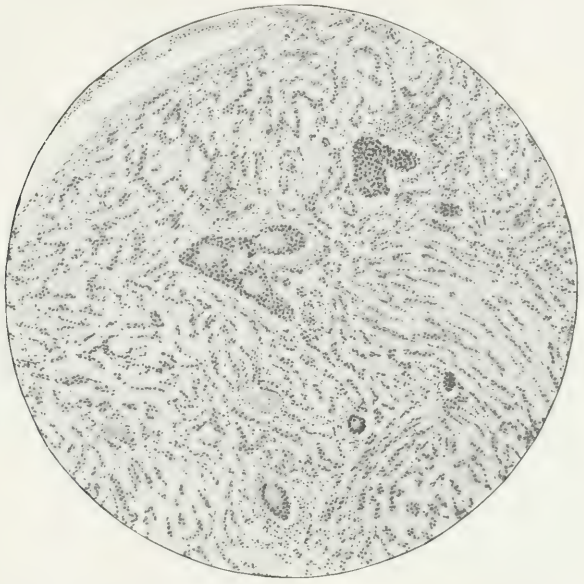


FIG. 3. Neuroblastoma: Low power drawing showing alveolar arrangement of cells and rosettes.

pink-staining tangle of fine fibrils, identical in every respect with the rosettes described by Wright and others. The stroma of the tumor was quite loose and delicate, but was everywhere distinctly seen. Rosette formation was a more striking feature of some of the large retroperitoneal nodes than of the inguinal node (Fig. 3). Indeed, in some of the sections almost all the tumor cells were thus arranged. In such places the stroma was very scanty. In a section of one gland, several areas of necrosis were

found, but in general both necrosis and hemorrhage were only exceptionally encountered.

Macroscopic metastases were noted only in the lymphatic and osseous systems, but microscopically they could be demonstrated, also, in the ovary, liver, spleen, and adrenals. In the ovary, there was a small but well-organized tumor nodule occupying less than a low power microscopic field. In the spleen,

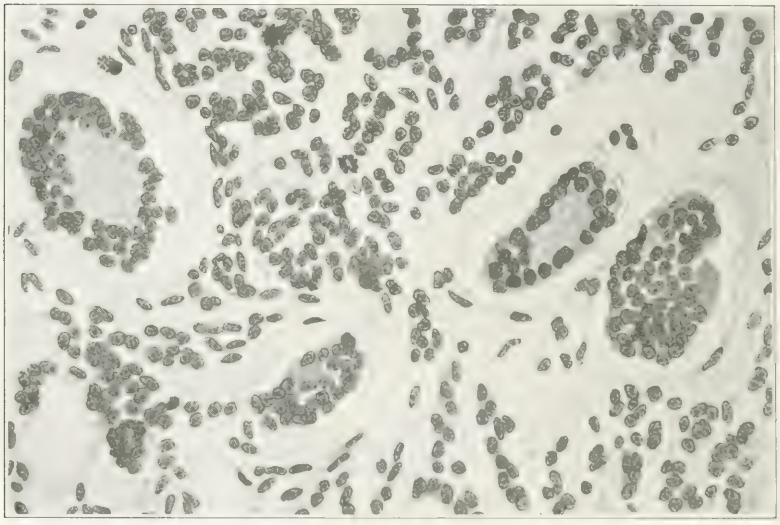


FIG. 4. Neuroblastoma: High power drawing showing morphology of cells, formation of rosettes with central mass of fibrils, and delicate connective tissue stroma.

there were no circumscribed tumor foci, but scattered everywhere through the pulp were small aggregations of tumor cells, arranged generally in rounded clumps or rosettes, which appeared to lie in distended sinuses without any supporting stroma (Fig. 4). The lymphoid follicles were nowhere invaded. In the liver, two types of foreign cells were seen: first, cells resembling lymphocytes with deep-staining nuclei and scanty cytoplasm lying between the cords of liver cells. These were few in number and never more than five or six together; they were prac-

tically identical in appearance with the blood-forming elements generally seen in the livers of congenital syphilitic infants, and represent, no doubt, an effort to extramedullary blood formation, demand for which was aroused through the extensive replacement of the bone-marrow by tumor. The cells of the second type, seen both in the liver and adrenal, were distinctly neoplastic. They lay free in the capillaries; and the groups were too small for any arrangement into ball-like aggregations or rosettes.

Specific neuroglia and fibroglia stains were applied to sections showing the most abundant fibril formation, but the results were uniformly negative. This is in accord with the experience of Wright and others.

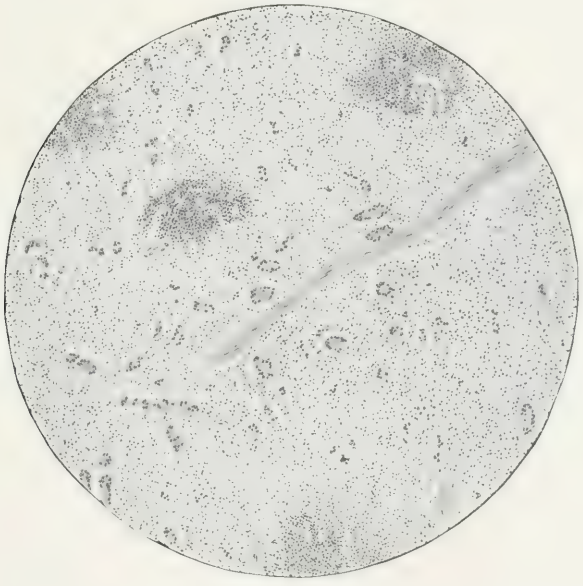


Fig. 5. Neuroblastoma: Metastasis in spleen. Note discrete groups of tumor cells growing in sinuses of pulp, with tendency to rosette formation.

Anatomical Diagnosis.—Neuroblastoma, arising in retroperitoneum; metastases in iliac, inguinal, mediastinal, axillary, and cervical lymph-nodes, spleen, ovary, and osseous system, especially pelvic bones, vertebræ and skull; groups of cells free in

capillaries of adrenal and liver; replacement of bone-marrow by tumor with extramedullary blood-formation in liver; anemia.

SUMMARY

This case presents all of the distinguishing histological features of the malignant neuroblastomata. The tumor cells are quite characteristic in their morphology and in the tendency toward an arrangement into alveoli and rosettes. There is abundant formation of fibrils which do not give the specific neuroglia or fibroglia standing reactions.

In the location of the primary tumor and in the distribution of metastases the case is, however, not so typical. The accompanying list of previously reported neuroblastomata shows that the primary tumor was found most often in the adrenals, which in our case were uninvolved, except for the presence of small groups of cells free in the capillaries.

It is also exceptional, according to this list, for the liver to be spared in cases where metastases are present, gross nodules being found in fourteen out of sixteen cases. In our case there were no macroscopic metastases in the liver, and microscopically only a few cells in the blood-vessels were found, as in the adrenals.

The present case is the first to be reported in which metastases were found in the spleen and ovary. These were not recognizable in the gross specimens and were found only in the routine microscopical examination of organs. It is quite possible that similar small metastases may have been overlooked in some of the previously reported cases where complete microscopic studies were not made.

Widespread metastasis in the lymphatic system and bones, especially the skull, as found in the present autopsy, appears to be a characteristic feature of the more malignant types of neuroblastoma. The seven cases of primary sarcoma of the adrenal with metastasis to the skull, reported by Hutchinson, very probably belong to this group.

Cases of Malignant Neuroblastoma

Author, Date	Age	Sex	Location	Metastases	Diagnosis
Dalton, 1885	6 weeks	M.	Left adrenal	Liver	Lymphosarcoma
Marchand, 1891	9 months	F.	Right adrenal	None	Neurocytoma?
Amberg, 1904	2 months	F.	Left kidney	Liver, right adrenal?	Malignant tumor of adrenal medulla
Bruck, 1904	14 months	F.	Right adrenal	Liver, pancreas, skull, lymph gland	Hemorrhagic round-cell sarcoma
Richards, 1905	2 weeks	M.	Left adrenal	Liver, lymph gland	Small round-cell sarcoma
Küster, 1905, Case I	14 weeks	?	Right adrenal	Liver, left adrenal	Glioma
Case II	34 years	M.	Left adrenal	None	Glioma
Lapointe & Lecene 1907	19 months	F.	Left adrenal	Lymph gland, mediastinum	Glioma
Alezais & Peyron, 1907	6 years	M.	Between rectum and sacrum	None	Parasympathoma
Schilder, 1909	7 days	F.	Right kidney attached to 2d left vertebra	None	Malignant glioma
Hecht, 1909	9 years	F.	Retroperitoneum	Adrenal, liver, lymph gland, duodenum	Lympho- or glio-sarcoma
Tileston & Wolbach, 1910	16 months	M.	Between right kidney and liver	Bones and lymph gland	Lymphoma?
Wright, 1910, Case I	1 day	M.	Both adrenals	?	Neurocytoma or neuroblastoma
Case II	Adult	M.	Hilum of lung	Liver	do.
Case III	?	?	Cerebellum	None?	do.
Case IV	16 months	F.	Multiple, retroperitoneum, skull	Mediastinum, liver, pelvis?	do.
Pick, 1912	23 years	F.	Uterus and broad ligament	Liver and lymph gland	Ganglioma embryonale sympathicum
Landau, 1913, Case I	8 months	F.	Retroperitoneum	Liver	Malignant neuroblastoma
Case II	2½ years	F.	Right adrenal	Liver, lymph gland, kidney bones	Malignant neuroblastoma
Case III	1 day	F.	Left adrenal, right adrenal	Liver, lymph, gland	Malignant neuroblastoma
Symmers, 1913	44 years	M.	Scapular region	None	Neuroblastoma
Anitschkow, 1913	4 months	F.	Retroperitoneum	Infiltration into spinal canal	Malignant neuroblastoma
Dunn, 1914	14 years	M.	Right adrenal	None	Neuroblastoma
Glomset, 1915	2 years	M.	Right adrenal	Liver, lymph gland	Neuroblastoma

Cases of Malignant Neuroblastoma—continued

Author, Date	Age	Sex	Location	Metastases	Diagnosis
Harbitz, 1915,					
Case I.....	3 years	?	In front of sacrum	None	Neuroblastoma
Case II.....	6½ years	?	Left adrenal	Liver	Neuroblastoma
Case III.....	Fetus	M.	Sacral region	?	Neuroblastoma

The cases of primary sarcoma of the adrenal reported by Parker, De Ruyter, Heaton, and Hutchinson probably belong here. They have not been included, however, because of incomplete data.

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Discussion:

Dr. Ewing: This case is peculiar in that it does not involve the adrenals. It is limited to the lymphatic system apparently, and in several respects it is different from the majority of adrenal neuroblastomata which tend to involve the liver. It might be well to add to the evidence already presented the results of specific stains, to determine whether definite fibrils can be demonstrated. I am inclined to think that neuroblastoma is not so rare as has been reported. I do not know whether Dr. Lambert has seen Dr. Symmers's case. We have another tumor in our laboratory which we have classified as a neuroblastoma, and I imagine that in the services of children's hospitals the country over, malignant tumors of this sort are still occasionally escaping recognition. I believe that Wright or Tileston and Wolbach have collected a number of cases which had been wrongly interpreted. It is probably true that certain tumors in children reported as lymphosarcomata are types of neuroblastoma of the adrenals. One of these groups, forming quite a series, was described by Pepper and Stengel some years ago.

Since a tumor of this nature may produce round cells, I think it wise that lymphosarcomata in children should in general be regarded with suspicion unless they are quite characteristic in distribution. Of course, with many indifferent round-cell tumors it may be impossible to recognize the histogenesis, but in my opinion, lymphosarcoma is the last diagnosis which should be made in children.

Dr. Moschcowitz: In connection with Dr. Ewing's suggestion that these tumors are probably more frequent than is generally supposed, I recall seeing abroad tumors demonstrated as gliomata, in which rosettes of an appearance identical with that described by Dr. Lambert were present. They are known as "Winterstein's rosettes." In all probability some of these tumors were neuroblastomata.

Dr. Lambert: This case is exceptional, though not unique, in not having its origin in the adrenal and in not showing extensive metastases in the liver. Among the twenty-seven cases which I was able to collect from the literature, there were eleven in which the adrenal was uninvolved; in four of these, the primary tumor was in the retroperitoneum, as in my case. Regarding the incidence of these tumors, I am inclined to disagree with Wright, who having seen four in one year, believes that the tumor is not an uncommon one, but is often overlooked. The growth presents such a striking clinical and pathological picture that it is difficult to understand how, in children especially, it could be overlooked or confused with any other type of neoplasm. Furthermore, several pathologists, including Symmers and myself, who have looked over a large number of tumors in several institutions, have been unable to find any other cases than those reported.

In making a diagnosis of neuroblastoma, special stains are of value only in a negative way, as I pointed out in my paper. Fibrils are abundant in most of the cases. They do not stain, of course, as neuroglia or fibroglia fibrils. Herxheimer, I believe, was able to show that they reacted as nerve fibers with the Bielschowsky silver method, which I have not used.

SARCOMA OF THE BREAST: A REPORT OF SIX CASES

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Sarcoma of the breast is a relatively infrequent neoplasm and though the older statistics give the usual proportion of sarcoma as 10 per cent. of all breast tumors, Rodman (1) places it at 2.7 per cent., while Bloodgood, in his experience, found it to be but 2 per cent. Though breast sarcomata have been described before the twenty-fifth year, such cases are infrequent. For the most part the tumors are encapsulated and usually they displace the mammary gland, tending to push it aside, rather than to infiltrate either it or the surrounding tissue. Broadly speaking, they are elastic, though the more cellular types and those in which hemorrhage or necrosis has occurred are cystic and fluctuating. As with carcinoma, excessive growth activity occurs during pregnancy. While all types of sarcoma have been recorded, the spindle-cell type is the more common. The life history of these tumors resembles closely the history of the mixed tumors of the parotid gland, and Wilms' view as to the similarity of their origin is generally accepted.

During the past five years on the various services of the German Hospital there have occurred 276 cases of tumor of the breast; of these, all but two occurred in females. The types of tumor were divided as follows: carcinoma, 68 per cent.; sarcoma, 2 per cent.; benign tumors (fibroma, adenoma, lipoma, etc.), 30 per cent. These percentages are approximately those given by others who have had several hundred cases in a series. The average age of the patients with sarcoma was forty-six years, and all were females. No etiological history of any definite value could be ascertained, though several patients gave the not unusual vague history of antecedent trauma. None of the cases could be traced at the present writing; hence the outcome

of operation can not be stated. In all of the cases a radical amputation was performed.

The first case of the series is a round-cell sarcoma which in the gross presented two fairly discrete nodules. The larger of these two nodules was rounded and sharply demarcated from the surrounding tissue, and measured 25 mm. in diameter. On section it was pearl gray in color, translucent, moist, and firm,

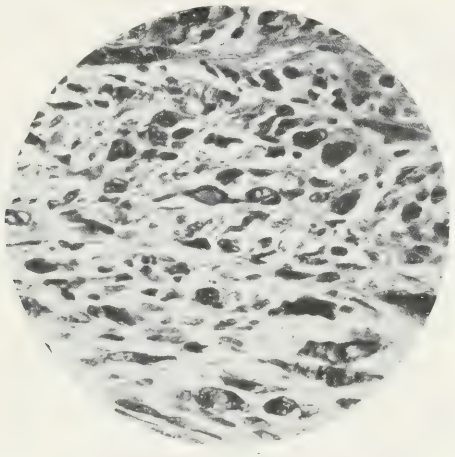

FIG. 1. Sarcoma of breast. Case 3.

but not hard. In consistency it was quite elastic, and there were some areas of hemorrhage demonstrable in the gross. The other nodule, situated adjacent to the nipple, was smaller and much more hemorrhagic. The nipple was inverted, but not retracted. Microscopically, the tissue was very homogeneous and was composed of small cells with relatively large vesicular nuclei. The cells were about twice as large as the red blood cells. Mitotic figures in every form were extremely numerous. The stroma was not very abundant and did not fall into distinct alveoli, and here and there were areas of necrosis and hemorrhage. No distinct capsule could be demonstrated. The axillary lymph-nodes did not show involvement in the process.

The second case, also a round-cell sarcoma, in the gross pre-

sented a rounded encapsulated mass of grayish-white color, of semi-fluctuating consistency, measuring 15 cm. in diameter. Section through the tumor showed quite extensive areas of hemorrhage, necrosis, and liquefaction. Microscopically, the tumor differed from the one described in the previous paragraph only in the much more extensive areas of necrosis, and in the presence of a well-developed capsule of connective tissue which was distinctly invaded by the growth. The axillary lymph-nodes showed no involvement.

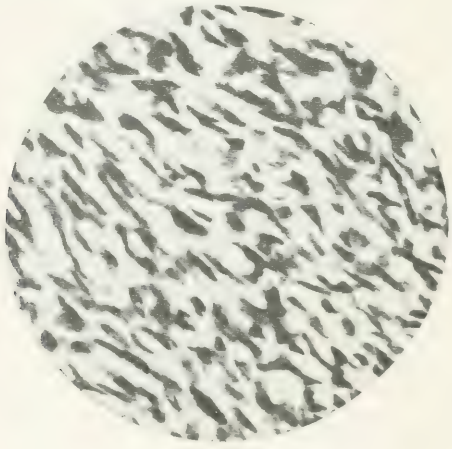

FIG. 2. Sarcoma of breast. Case 4.

The third, fourth (Fig. 1 and 2) and fifth cases in the series were typical spindle-cell sarcomata. In the gross, the tumors were usually globular, of soft consistency, and bounded in all three cases by a soft, delicate capsule which was fairly smooth and definite. Central degenerated areas of gelatinous consistency were common. The largest of the growths measured 10 by 6 cm. in diameter. Histologically, the neoplasms resembled the usual picture of spindle-cell sarcoma in other locations. Lymph-node involvement could not be demonstrated.

The sixth case of the series was a fibroliposarcoma (Fig. 3). The growth presented itself in the gross as a soft, irregular mass, measuring 3 cm. in diameter. Its appearance suggested a rather

fibrous lipoma. Histologically the tumor consisted of intertwining bundles of rather closely packed, elongated, spindle cells separated by small amounts of fibrillar and granular intracellular substance. Amidst these bundles were found scattered areas of adipose tissue, but no glandular or epithelial structures. There were no extensive areas of necrosis. No distinct capsule could be made out. Mitotic figures were numerous. Glandular involvement could not be demonstrated.

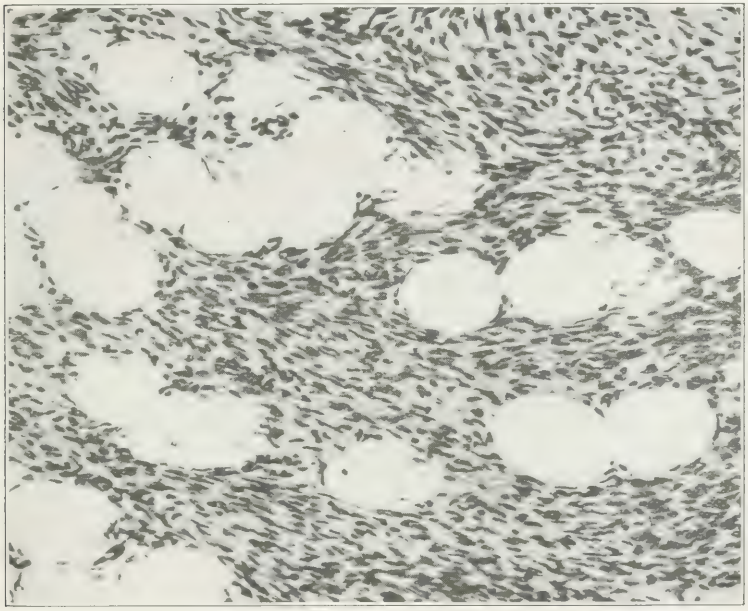

FIG. 3. Fibroliposarcoma of breast. Case 6.

The interesting features of the cases are the rather advanced age for sarcoma development; the more or less encapsulated character of the growths, and the absence of any distinctive breast tissue in the neoplasms. It is interesting also to note that the percentage of sarcoma incidence in this series of breast tumors corresponds to the figures given by Rodman and by Bloodgood, rather than to the higher percentages quoted by various continental writers.

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Discussion:

Dr. Wood: I think the high figures which have been given abroad are due to the fact that many of the cases considered as sarcoma are really very cellular intracanalicular fibromata, which the older French pathologists were in the habit of describing as sarcomata. I have seen one specimen of that type—a rather large, rapidly growing intracanalicular fibroma with many dividing cells and also a great many large dividing fat cells, which was diagnosed as sarcoma by many pathologists at that time (some twenty years ago). I was then acting as house surgeon at the hospital and followed the case with some interest; no recurrence taking place, the tissue was reexamined. It did look very much like a sarcoma, and I think any one would, without a careful search of the tissue, make that diagnosis; a thorough study of the sections, however, showed that the tumor was only a very rapidly growing, edematous, intracanalicular fibroma.

It is curious how infrequent chondrosarcoma of the breast is in human beings, although it is so common in dogs. I have seen only one case of this type in a human being, and there are but few cases reported in the literature of the subject.

Dr. Ewing: Six well-attested cases of sarcoma of the human breast are rather a large number in the experience of one institution. I have no doubt, however, that these cases are genuine. It seems to me that some of them have gross features which are rather distinctive and separate them in all probability from forms of carcinoma. Nevertheless, I feel that one ought to be very circumspect in making a diagnosis of sarcoma of the breast, and that it should be made only after a very thorough study to determine whether the growth may not be a round-cell carcinoma. However, if a diagnosis of sarcoma of the breast is made, I think we are under obligation to give something more than the simple diagnosis of sarcoma, the particular type ought to be pointed out, and some indication of the histogenesis ought to be given. There are some rather definite forms of mammary sarcoma; for instance, the sarcoma developing in an intracanalicular fibroma or the malignant analogue of intracanalicular fibroma is a well-recognized, more or less encapsulated, somewhat cauliflower form; and I should judge that one or two of Dr. Rohdenburg's cases were of that type. The diagnosis of round-cell sarcoma I think is not very helpful. Is the tumor a lymphosarcoma, primary or secondary, or a metastatic sarcoma? I have never seen lymphosarcoma of the breast that was not secondary. Metastatic round-cell carcinoma in the breast is not very infrequent. The last specimen I saw was from a round-cell carcinoma of the ovary, and here the first tumor recognized was a bulky mass in the breast. I do not think at present that a diagnosis of round-cell sarcoma of the breast is one that should be made; one ought to go further and state the probable origin. I have seen two cases of mammary sarcoma.

one in a male and one in a female breast. The former was a neurogenic sarcoma; the latter, which occurred during the past week at the Memorial Hospital, was a perfectly definite large spindle-cell sarcoma apparently arising from the stroma of the breast. I have been impressed by the great number of round- and spindle-cell sarcomata which come to my hands, and which upon a thorough study of a series of sections show here and there alveolar structures, which we used to call alveolar sarcoma, but which we now know, and have long known, belong in almost all instances in the group of carcinomata. On the whole, I do not feel a great deal of enthusiasm about any form of sarcoma of the breast that does not show some definite origin from a known structure and that can be confused with atypical carcinoma.

A METHOD FOR STANDARDIZING THE READING OF THE WASSERMANN REACTION, WITH A DEMONSTRATION

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Standardization of Reading Desirable.—It seems highly desirable that the readings of the Wassermann reaction should be so standardized that the blood of a given patient would always receive the same reading in the same laboratory and in different laboratories, unless there is a change in the blood to justify a different reading. Unfortunately, at the present time, this is not always the case. There are Wassermann tubes in which the reaction is so near the midpoint between two readings that any serologist may experience the greatest difficulty in deciding whether it should be considered a one plus or a two plus, or whether, in another case, it should be a two plus or a three plus.

Variations in the readings of the Wassermann reaction which do not correspond to changes in the sera are particularly disturbing in the cases of patients who are undergoing treatment. There is a distinct need, therefore, for some method whereby the weakest reaction as well as the strongest may be read with uniformity; and the purpose of this paper is to suggest a method for standardization.

The Problem.—The problem in reading the Wassermann reaction is to determine the fate of the sheep's corpuscles that are added to the tubes as indicators. Each Wassermann tube, after all of the reagents are added, contains 1 per cent. of sheep's corpuscles. These corpuscles subsequently may be found entirely destroyed, partially destroyed, or not at all destroyed. If they are completely destroyed, the reaction is negative. If they are completely preserved, the reaction is the strongest positive. If they are partially preserved and partially destroyed, intermediate readings must be given. These readings may be in percentages or they may be given by assigning different numbers of pluses according to the number of corpuscles preserved.

Problem Simplified.—In order to simplify the problem, let us, for the time being, decide to give a reading to each tube, these readings to be either four plus, three plus, two plus, one plus, or negative. Two of the readings, namely, the four plus and the negative, we can determine readily by definition. We can read as four plus all tubes in which, after centrifugalization or after the corpuscles have been allowed to settle, there is no apparent hemolysis or only the barest trace of hemolysis. As negative, we read all tubes in which there is complete hemolysis or the barest trace of unhemolyzed cells. Tubes in which the corpuscles are partly preserved and partly hemolyzed are to receive the readings one, two, and three plus according to the number of corpuscles preserved. The real problem then is to distinguish sharply between a one plus and a two plus, and between a two plus and a three plus.

One and Two Plus Distinguished.—An observation was made nearly three years ago which suggests a way to distinguish between the one plus and the two plus. In reading the Wassermann tubes late at night, the tubes were held between the eye of the observer and the unfrosted bulb of an incandescent electric light. It was then observed that in the negative tubes the electric-light filaments could be seen with the greatest distinctness. When tubes which contained corpuscles that were almost completely hemolyzed were thoroughly shaken, it was seen that

the electric-light filaments could still be made out, although somewhat hazily. The larger the number of unhemolyzed corpuscles present in the tube, the more difficult it was to make out these filaments, until a point was reached where they were completely invisible. Here, then, apparently was a way to distinguish sharply two readings, provided standard conditions of light, and tubes of uniform diameter, were employed. The tubes used measured 1 cm. inside diameter. If, after the tubes were shaken thoroughly, the filaments of the electric-light bulb could still be made out through the fluid, the reading was one plus. If, however, the reaction was weak and the filaments could not be seen, it was regarded as two plus. This distinction between the one and the two plus was adopted and has been used with great satisfaction in reading some 24,000 Wassermann tubes.

Precautions.—If the readings are to be uniform, the following precautions must be taken:

1. In order to distinguish between one plus and two plus, the size of the tube should be 1 cm. inside diameter. If the Wassermann reaction is carried out in tubes that are not this size, it is necessary only to have at hand a few standard size tubes into which the fluid, after being shaken, can be poured and examined.

2. The light employed should be of moderate and comfortable intensity. A 110-volt, 25 to 40 watts, unfrosted Mazda bulb is satisfactory, but other lights can be used.

3. The incandescent filaments should be observed through a small hole in some opaque object interposed between the eyes and the lamp to shield the eyes from the excess of light. The top or bottom of a small pasteboard box with a hole, 1×0.5 cm., answers the purpose admirably.

4. An excess of light from sources other than the electric bulb must also be kept out of the eyes if the readings are to be uniform and exact. If one makes the readings in the daytime, facing windows through which a great deal of light is coming, it is well to pull down some or all of the shades.

5. To make the reading, the tube should be thoroughly

shaken and held close to one eye; the other eye may be shaded by the other hand. In order that the eye may be fixed on the filaments and not on the tube, the tube should be kept so close to the face that it touches or almost touches the cheek or forehead.

6. It is convenient and advisable when making readings (1) to be comfortably seated at a distance of 18 to 24 inches from the incandescent light, and (2) to look first through the test-tube above the part containing the fluid to be examined, to make sure that the incandescent filaments can be easily seen, before raising the tube and attempting to see the filaments through the fluid in the tube.

Accuracy.—If the above precautions are taken, the method here suggested seems to be extremely accurate in testing weak reactions, for in this way one can easily distinguish between 9 per cent. of 1 per cent. of preserved corpuscles and 10 per cent. of 1 per cent.

Two and Three Plus Distinguished.—In order by means of electric-light filaments to distinguish sharply between a two plus and a three plus reaction, it is necessary to see the filaments through fluid less than 1 cm. in thickness. After numerous trials, a thickness of 3 mm. was decided upon, and a pipette which had this as a maximum diameter was constructed. This pipette was small enough to be inserted readily into tubes of an internal diameter of 1 cm.

It was found desirable to construct this pipette so that it had a transparent flat front and back cut from microscopical slides, by slitting them longitudinally into rectangles measuring 7.5 cm. $\times$ 0.5 cm. These rectangles were so fastened together that from a distance of 3 mm. above, they sloped gradually towards each other until at the bottom they were only 1.5 mm. apart. The calculated distances between the front and the back were maintained by inserting between them accurately cut wedges of glass. By means of adhesive plaster these wedges were fastened firmly at the sides to the front and back of the pipette. The adhesive plaster was subsequently soaked in hot paraffin to give the pipette greater stability and to make it watertight. For con-

venience in using and handling, the pipette was lengthened by joining it at the top to a cylindrical piece of glass tubing 12 cm. long. A rubber nipple on the upper end of this glass tubing made it easy to draw fluid into the lower measured portion of the pipette and to expel this fluid again after the reading had been made.

Using the Pipette.—The tube to be examined is first shaken gently but thoroughly so as to distribute the corpuscles evenly throughout the fluid. The pipette is next inserted into the test-tube and the fluid drawn into the measured portion. The pipette is then pulled up until only the tip at the bottom remains in the test-tube to catch possible leakage. The suspension in the pipette is then brought close to the eye and the observer tries to see the incandescent filaments through the fluid in the pipette. If the reading is a two plus, he will be able to see the filaments readily through the lower part of the pipette and as the pipette is moved downward in front of the eye the filaments will still be seen, although perhaps with a little difficulty, quite to the top of the flat part of the pipette. If the reading is a three plus, the filaments will disappear before the top is reached.

A pipette could be constructed to distinguish quickly between a three plus and a four plus, but experiments indicate that the readings with pipettes of this kind are most delicate in suspensions containing fewer corpuscles than are present in the strong reactions. When, therefore, there is doubt between a three plus and a four plus reaction, the writer believes that the separation can be made with greater certainty and accuracy by the observation of an unmistakable trace of hemolysis in the three plus tubes after sedimentation or centrifugalization.

Reading by Percentages.—It is possible to read the Wassermann reaction and report it according to the percentage of corpuscles that remain unlaked in the different tubes, but this method is not recommended. Several reasons may be mentioned: (1) It does not seem necessary to report the readings in this way; (2) the readings by percentages would be more time-consuming; (3) the readings by percentages would be made with

Table Showing How Standard 1 Per Cent. Solution and Suspensions Were Combined.

	Tube Number														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Per cent. corpuscles preserved	100	99	98	97	96	95	94	93	92	91	90				
	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.				
Amount 1 per cent. suspension	1.0	.99	.98	.97	.96	.95	.94	.93	.92	.91	.90				
	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.				
Amount 1 per cent. solution*..	.00	.01	.02	.03	.04	.05	.06	.07	.08	.09	.10				
Reading..	– – – +	+ · · +	· · ! +	+ · · +	+ + +	+ ·	+++	+++	+++	+++	+++				
Per cent. corpuscles preserved.	100	90	80	70	60	50	40	30	20	10	0				
	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.				
Amount 1 per cent. suspension	1.00	.90	.80	.70	.60	.50	.40	.30	.20	.10	.00				
	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.				
Amount 1 per cent. solution*..	.00	.10	.20	.30	.40	.50	.60	.70	.80	.90	1.00				
Reading..	– – · +	+ + +	· · +	· · +	· + + + +	· +	+ +	+ +	+		0				
Per cent. corpuscles preserved.	14	13	12	11	10	9	8	7	6	5	4	3	2	1	0
	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.
Amount 1 per cent. suspension	.14	.13	.12	.11	.10	.09	.08	.07	.06	.05	.04	.03	.02	.01	.00
	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.
Amount 1 per cent. solution*...	.86	.87	.88	.89	.90	.91	.92	.93	.94	.95	.96	.97	.98	.99	1.00
Reading.....	+ · ·	· · ·	· · ·	· · ·	+	+	·	·	+	–	–	·	0	0	0

* The 1 per cent. solution of sheep's corpuscles was prepared by making first a 2 per cent. solution of the corpuscles in distilled water, then adding an equal volume of 1.8 per cent. salt solution which reduced the solution to 1 per cent. and at the same time made the fluid isotonic with that in which the corpuscles were suspended. Both suspension and solution were made from washed corpuscles packed for 10 minutes at about 2,500 revolutions per minute.

varying degrees of accuracy for they are most delicate where the corpuscles are few. It is as easy, for example, to distinguish 9 per cent. of 1 per cent. of corpuscles from 10 per cent. of 1 per cent. of corpuscles as it is to distinguish 90 per cent. of 1 per cent. from 100 per cent. of 1 per cent.

In order to read by percentages the writer found it necessary to do two things: (1) To construct a series of hollow prisms of varying size; and (2) to prepare standard suspensions and solutions of 1 per cent. of sheep's corpuscles and then combine these so as to give a series of tubes, resembling as nearly as possible the contents of Wassermann tubes, but differing from these in that the number of corpuscles present in each tube was definitely known. The table below shows what standard tubes were usually set up and how the solution and suspension of corpuscles were usually combined to make these standards.

The hollow prisms were graduated by pouring into them the contents of the standard tubes and marking on the sides of these prisms the point at which the electric-light filaments disappeared. In each case the percentage of corpuscles that caused this disappearance was also noted. After the prisms were standardized and had been checked a number of times to see that they would distinguish varying percentages of corpuscles, it was strikingly evident that this method was most delicate where the corpuscles were few. Under these conditions a difference of 1 per cent. of 1 per cent. of corpuscles could be distinguished as easily as a difference of 10 per cent. of 1 per cent. where the corpuscles were more numerous.

Value of Readings.—The value of the readings of the writer was determined by using standard suspensions of solutions of corpuscles and testing these by the tube and the pipette method already described. The result is indicated below:

++++ equals 97 per cent. to 100 per cent. of preserved corpuscles.

+++ equals 47 per cent. to 96 per cent. of preserved corpuscles.

++ equals 11 per cent. to 46 per cent. of preserved corpuscles.

+ equals 3 per cent. to 10 per cent. of preserved corpuscles.

0 equals less than 3 per cent. of preserved corpuscles.

These readings are quite arbitrary, but are useful and have seemingly given satisfaction in a service where approximately 1,000 complement fixation tests are made each month. If serologists decide to use the principal here suggested, they can make the point of separation between one, two, and three plus whatever they choose by varying the size of the tubes or the pipettes employed.

Wide Applicability of Principle.—It seems to the writer that the principle here suggested may have a rather wide applicability, especially if it be employed in connection with serial dilutions. It may be used for standardizing the number of corpuscles present in the suspensions used in the Wassermann reaction, and may, perhaps, also be used in standardizing bacterial emulsions and in estimating the amount of various precipitates.

Objections Answered.—It is so easy to raise objections against something that has not been tested and it is also so easy to be satisfied that the untested objections are important that I venture to answer some of those that may be urged against this method.

1. Some may think that they have so many Wassermann reactions to read and so many other things to do, that they will not have time to make readings by this method.

In reply, I may say that this method was worked out largely because of the necessity of saving time and, in my case, following the directions here outlined has saved time. The majority of the reactions sent to me are negative and are read after a careful glance. Of the rest, many are four plus reactions and these are also usually read by inspection in accordance with the definition here given. The remaining readings, the one plus, the two plus, and the three plus, are not numerous and the majority of this small number will be far enough removed from

the border-line between the one plus and the two plus, or the border-line between the two plus and the three plus, to occasion no difficulty. The border-line tubes are really difficult to read and here is where the method suggested is to me of great value, for I can now read with certainty, in four to ten seconds, tubes the reading of which I formerly had to guess at, no matter how long I spent in examining them.

2. It may be said that the system is not worked out for the quantities in use in different laboratories, and that one cannot afford to change his way of doing the Wassermann reaction, nor to buy a large number of standard tubes.

In reply, I admit that it is quite true that the Wassermann reaction is done differently in different laboratories, that different size test-tubes are employed, and that the final quantity of fluid in these tubes may be one tenth, one quarter, one half, or some other fraction of the original Wassermann reaction, but the method here suggested works admirably with quantities as small as one tenth of the original and there seems to be no inherent difficulty in employing it easily with all the other quantities. In making the distinction between the two plus and the three plus, a pipette is employed which is as easily used with these larger quantities as it is with the one tenth quantities. In making the distinction between the one plus and the two plus, there is a seeming difficulty for those who employ tubes that are larger or smaller than 1 cm. inside diameter. This difficulty, however, is not great, for it is probably easy for any laboratory to have on hand a few clean dry tubes of clear glass with an inside diameter of 1 cm. When there is doubt between a reading of one plus and two plus, the shaken contents of weakly positive Wassermann tubes can be poured into these standard tubes, held up to the light, and read in a few seconds.

3. Others may say they have no 1 cm. tubes and have no time to make a pipette.

Even in this case readings which closely approximate those given here can be obtained if tubes which are nearly 1 cm. in inside diameter are used. These tubes should be standardized

by employing standard solutions in which the number of corpuscles present are known. By using these solutions one can determine the highest percentage of corpuscles through which the electric-light filaments can be seen. This will probably be 9, 10, or 11 per cent. of 1 per cent., according to the size of the tube employed and the light which enters the eyes of the observer. This percentage can be taken then as the standard for separating one plus from two plus readings. Tubes of the same size should be chosen, marked, and employed for these determinations.

The same tubes can be used for separating the two plus from the three plus reactions if the fluid to be examined is diluted until the electric-light filaments can just be seen. Knowing the percentage of corpuscles through which the filaments can be made out, and knowing the dilution that was necessary to make the corpuscles visible, the percentage of corpuscles present in the unknown tube can be calculated. If this number is above 46 per cent. the tube can be read as a three plus. If it is 46 per cent. or less it can be read as a two plus.

An example in which the Wassermann tubes are diluted will make this method clearer. We will suppose that one has test-tubes the inside diameter of each of which is one centimeter, and that one is working with one tenth the original Wassermann quantities. In this case each of the Wassermann tubes, after all the reagents are in, contains about 0.5 c.c., the exact amount depending on the technique employed. If the dilution method is employed, one should determine exactly what the final quantity is, and should then add to the tubes that are on the border-line between two plus and three plus enough normal salt solution to bring the total up to 2.3 c.c. The tube should be shaken thoroughly, and examined before an electric light bulb in the manner already described. If the electric light filaments can be seen through the diluted corpuscles, it shows that the tube previously contained about 46 per cent. or less of 1 per cent. of corpuscles. By dilution this has been reduced to 10 per cent. or less of 1 per cent. and the reaction should be read as two plus. On the other hand, if the filaments can not be seen through the diluted cor-

puscles, it shows that the reading should be higher than a two plus. In these border-line tubes it would be three plus.

If one is using larger quantities than one tenth the original Wassermann, one can easily differentiate tubes on the borderline between the two plus and the three plus by shaking them gently but thoroughly, and then pipetting 0.5 c.c. of each suspension to be tested into a tube having an inside diameter of 1 cm., adding to it 1.8 c.c. of normal salt solution, shaking, and examining before an electric light bulb as already described. If the filaments can be seen the reading is a two plus, and if they can not be seen, it is a three plus.

Conclusion.—If the reading of the Wassermann reaction is to be standardized so that readings of the weaker reactions will be the same in different laboratories, it seems probable that either the method here described or some similar one will have to be employed. It is scarcely to be hoped that the method and readings here suggested will be adopted without change and the writer would welcome helpful suggestions from any serologist.

Discussion:

Miss Prigosen: I should like to ask how permanent the standard solutions are, and how soon the Wassermanns are read.

Dr. Terry: I do not think the standard solutions are permanent. These were made to-day for to-night. The Wassermanns are read twice by us, once the day they are done, and again the next morning. My technician makes the first reading, but I make the final reading myself.

FOCAL NECROSIS OF THE ADRENAL; WITH REMARKS UPON ACUTE ADRENAL INSUFFICIENCY

ELI MOSCHCOWITZ, M.D.

Two cases were described. The first occurred in a man, aged forty-one, who died three days after nephrectomy for a pyonephrosis. The most prominent symptoms were a subnormal temperature and slow respiration and pulse. At the

autopsy, the right adrenal showed a number of sharply defined focal necroses scattered throughout the cortical zone; the cells were profoundly degenerated, and there was abundant polymorphonuclear infiltration within and surrounding the necrotic area. The adrenal was moderately hemorrhagic, and many of the capsular vessels showed a recent thrombosis. (The gland was enmeshed in an extensive hematoma.) The thromboses were most abundant in those portions of the cortex where the necroses were most numerous.

The second case occurred in a child who was sick for a long time with an abdominal ascites and chyluria. Three days before death a streptococcemia developed, from which the patient died. At autopsy, in addition to bacterial emboli in the spleen, pancreas and kidney, emboli were found abundantly just beneath the capsules of both adrenals. Sometimes only the embolus was found; at other times the emboli were surrounded by a polymorphonuclear infiltration. In these foci the adrenal cells were degenerated.

The literature was reviewed to determine the incidence of acute inflammatory lesions in the adrenal, and it was found that these were most common in some of the infectious diseases, notably in diphtheria, variola, typhoid, tetanus, pneumococcus infections, dysentery, and streptococcus infections. Experimentally they have been obtained after injections of some of the pathogenic bacteria, for example, the Klebs-Lœffler bacillus, B. pyocyaneus, B. tetanus, B. anthracis, the pneumococcus, streptococcus, etc.

The following varieties of lesions have been found associated with infections: (1) parenchymatous degenerations; (2) edema; (3) hemorrhages; (4) leucocytic infiltration; (5) focal necrosis; (6) miliary bacterial emboli; (7) abscesses. Often bacterial emboli in the adrenals are the only evidence of a general infection.

Pathogenetically, the lesions in the adrenals may result either from general toxic action or from bacterial emboli. In the first case here reported, another cause was possible, namely, thrombosis of the capsular vessels (infarcts).

The clinical aspects of acute adrenal insufficiency, to which considerable attention has been paid, were reviewed. It was shown how diverse and conflicting the symptoms and signs that have been ascribed to this lesion are, and that, moreover, such symptoms and signs do not correspond with our present knowledge of the physiology of the adrenal. In other words, it is not believed by the author that the diagnosis of acute adrenal insufficiency is at present justified.

CONDITIONS OF THE EPIDIDYMIS SIMULATING CARCINOMA

A. P. STOUT, M.D.

(*From the Laboratory of Surgical Pathology, Presbyterian Hospital, New York*)

In many surgical conditions it is often difficult for the microscopist to distinguish a chronic inflammatory from a neoplastic condition, because of the extensive epithelial hyperplasia which may occur incidental to chronic inflammation. This hyperplasia has been noted in sections from the margin of ulcers, from epithelial-lined canals, and from many of the tubular glands.

The epididymis and the vas deferens are often involved in chronic inflammation, the common causes of which are tuberculosis, gonorrhea, and syphilis. Aside from these, however, there are supposed to be other chronic inflammations, the source of which cannot be ascertained, and these are passed over in most of the recent text-books on pathology without any descriptive details. However, in the 1886 edition of Ziegler's *Lehrbuch der pathologischen Anatomie* (Vol. II, 895) there is an account of the histology of cryptogenic chronic inflammation, of which the following is a rough translation: "In the epididymis the distorted ducts appear at times like the cords and masses of cancer building. Under the microscope one can see atypical epithelial

growths and the cell cords are increased in number. Sometimes the epithelial cells produce mucus so that mucous cysts occur. The canals may be filled with desquamated and fat-swollen cells." As may be imagined from reading this description, pitfalls exist for the unwary in differentiating chronic inflammation from carcinoma when the two conditions so closely approximate one another under the microscope.

The first two of the following cases illustrate this difficulty. They are on the border-line between malignant epithelial neo-

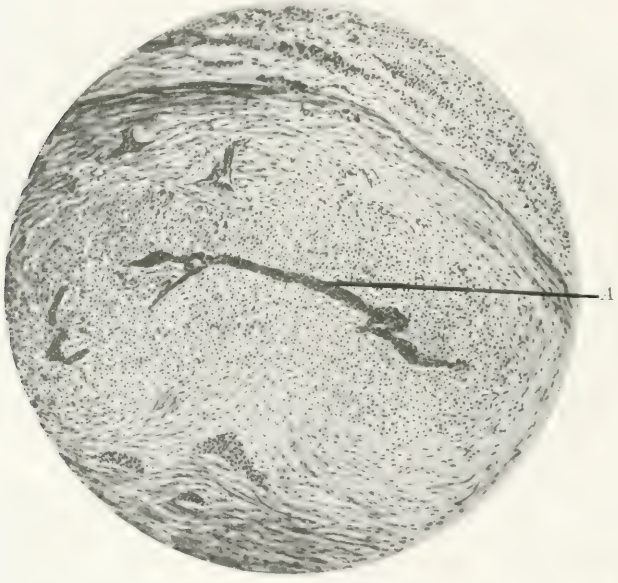

FIG. 1. Case I. *Drawing.* *A*, Strand of epithelial cells occupying site of epididymis tubule.

plasm and hyperplasia of the duct epithelium due to chronic inflammation. In the first case some of the tubules are tremendously dilated and their lumina filled with large, pale-staining, epithelial cells. Others have no lumen at all but are represented by a slender strand of epithelial cells which are smaller in size and somewhat more deeply stained (Fig. 1). The latter phase, which has been attributed to atrophy following prolonged irri-

tation, is the one which superficially simulates alveolar carcinoma (Fig. 2). This case is undoubtedly due to chronic inflammation, possibly of gonococcal origin, since there is a previous

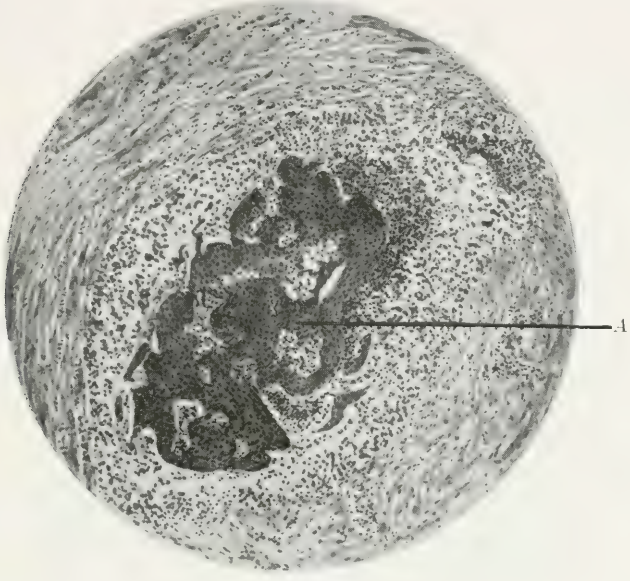

FIG. 2. Case I. *Drawing. A,* Mass of hyperplastic epithelial cells filling lumen of vas deferens.

history of gonorrhea. There was, however, no history of any acute attack of epididymitis and examination of the blood serum shortly after the operation gave a negative gonococcus complement-fixation test.

In the second case, the tubules are represented by masses of epithelial cells which are large and deeply stained, and show occasional mitotic figures. The outline of these masses is wavy and ill-defined (Fig. 3). Here we are dealing with a picture which, if it appeared elsewhere in the body, would without question be called carcinoma. Indeed, several pathologists to whom these slides were submitted made an unqualified diagnosis of carcinoma. It is well known, however, that in the breast, for instance, one may have a hyperplasia of the epithelium lining the

acini and ducts, thus giving a picture as to the true interpretation of which no group of pathologists can come to a unanimous decision. Since there has been a disagreement about the

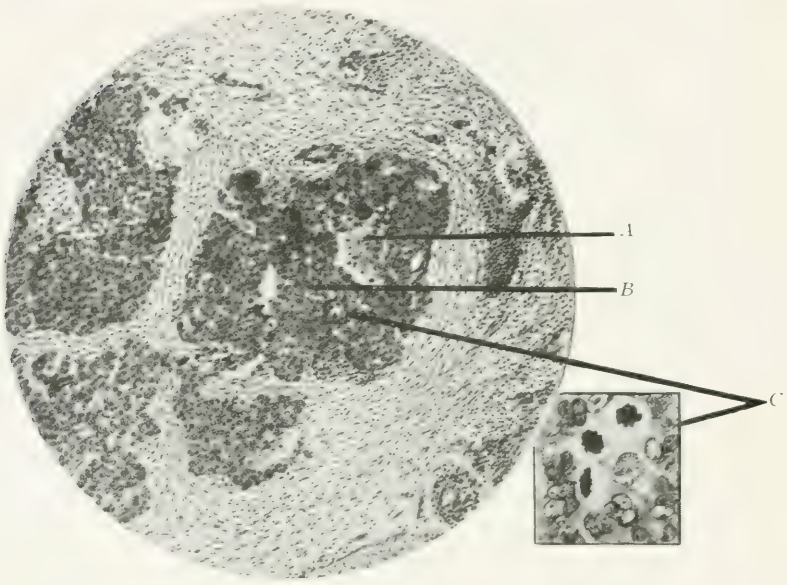

FIG. 3. Case II. *Drawing.* *A,* Degenerated epithelial cells; *B,* Mass of epithelial cells occupying site of epididymis tubule; *C,* Small square, shown also in high power, of cells including three mitoses.

diagnosis in this case, it is reported in order to emphasize the existence of a border zone between the microscopic pictures of chronic epididymitis and carcinoma.[1]

The third case records a mistake in diagnosis from a quick-frozen section stained with thionin at the time of operation and the reason for the mistake. Briefly, the sequence of events was as follows: At operation the surgeon found what was thought to be a mass composed of carcinomatous lymph-nodes blocking the common and cystic ducts from the outside and adherent to the hepatic flexure of the colon. The patient had a nodule in

[1] Unfortunately since this patient lives out of town, it has been impossible to ascertain anything about his present condition beyond the fact that he was alive nine months after his operation.

his scrotum which was attached to the lower pole of the epididymis. The existence of this had been unknown to him, and it was discovered only during the routine physical examination on admission to the hospital. It was excised and a quick-frozen section was made in the belief that it might be the primary tumor of which the mass about the common duct was a metastasis. The section showed interlacing strands of smooth muscle and connective tissue, and, in addition, a number of irregular tubules

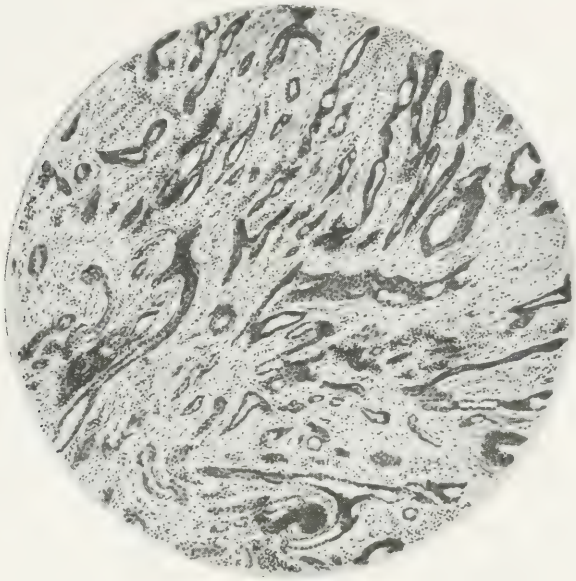

FIG. 4. Case III. *Drawing.* Showing vessels lined with hyperplastic endothelial cells. From surface of epididymis tumor.

lined with prominent swollen cuboidal cells (Fig. 4). The picture was apparently that of adenocarcinoma, and this diagnosis was made. Five days afterward the man died of post-operative pneumonia and autopsy showed that he had a primary adenocarcinoma of the common duct. Further sections of the mass in the epididymis were made and stained with Van Gieson's stain and with hematoxylin and eosin. These demonstrated that what had at first been taken for epithelial-lined tubules were in reality

blood or lymphatic vessels lined with swollen prominent endothelial cells. The rest of the tumor was composed entirely of smooth muscle and connective tissue without any of the tubules of the epididymis or signs of chronic inflammation. There seems little doubt that the mass was a fibromyoma. Incidentally, a hasty glance over the literature has not revealed any other recorded cases of fibromyoma of the epididymis.

Histories

Case I. H. O., Austrian, 37, married, presser. Admitted to the Presbyterian Hospital, October 5, 1916.

Present Illness: Sixteen days before admission the patient noticed that his left testicle had become three times as large as usual and was soft and painful, especially when touched. This lasted for three days; six or seven days after the onset it became stony hard, and remained the same size and consistency.

Past History: At seven years of age he jumped and his left testicle hurt him for one day. At thirteen, he hit his left testicle, causing pain and swelling for twenty-four hours. At eighteen, the left testicle became large and painful spontaneously. It was normal again after two days. Gonorrhea when thirty and thirty-five, but no trouble with testicles at those times. Malaria in Jamaica one year ago, otherwise history is negative.

Family and Personal History: Nothing noteworthy.

Physical Examination of Surgical Condition: The left side of the scrotum was swollen and contained an oval mass measuring 3×2 inches. The testicle was of normal size and could be seen outlined on the middle of the anterior surface of the mass, which was made up largely of the epididymis. The globus minor was of cartilaginous hardness, the globus major large but softer. The vas was thickened and beaded. By rectum, the prostate was of normal size. In the region of the seminal vesicles were two small, firm, non-tender nodules. Physical examination otherwise was negative. Urine, sputum, and prostatic fluid were negative for tuberculosis. The Wassermann reaction was negative. *X*-ray of chest was clear. Urine showed a few pus cells and Staphylococcus aureus on culture.

Operation: Epididymectomy by Dr. A. V. S. Lambert, October 16, 1916. The epididymis was enlarged, hard, and nodular, and there were nodules extending at various intervals up along the vas as far as it could be made out. The tunica vaginalis was distended with clear fluid. The soft structures about the testicle were edematous and adherent. The epididymis was adherent to the testicle at various points, but was dissected free without a great deal of difficulty.

Pathological Examination: The specimen consisted of the epididymis and tunics, and measured $5 \times 3.5 \times 2$ cm. The segment of vas attached to it was 10 cm. long. The epididymis was much swollen and reddened and the

tunics were at least 8 mm. in thickness. There was no caseation or necrosis of any kind in the specimen. The vas was irregularly thickened, measuring 4 mm. in diameter near its base.

Microscopic Examination: Sections from the epididymis showed that the glandular mass was largely destroyed. What remained was undergoing degeneration and was infiltrated with many round cells. At several points were small interlacing strands of epithelial cells growing in small groups. The same type of epithelial growth had replaced the mucosa and lumen of the vas at a point about 1 cm. from the epididymis. Another section of the vas, at its junction with the epididymis, however, showed an intact lumen and mucosa. The stroma of the epididymis was edematous and infiltrated with many round and polymorphonuclear leucocytes. The tunica vaginalis showed edema, thickening, and fibroblastic reaction.

Post-Operative Course: This was uneventful except for superficial infection of the operative wound, and the patient was discharged from the hospital on the fifteenth day. After leaving the hospital the patient had his blood serum tested for the gonococcus complement fixation with negative result. October 15, 1917 (one year after operation), the patient was working as a window-cleaner; no longer wore truss; and had perfect health. Left testicle was the same size as the right testicle and epididymis together. Nodules in the region of the seminal vesicles were unchanged.

Case II. H. S., American, 39, clergyman. Admitted to the Roosevelt Hospital, February 8, 1917.

Present Illness: Six weeks before admission, the patient had a dragging pain in right testicle which was tender to touch, and two weeks later noticed lump in back part of right testicle. A suspensory relieved the pain, but the lump kept increasing in size.

Past and Family History: Unknown.

Physical Examination: Revealed a hard swelling of the right epididymis, chiefly in the globus major. Wassermann reaction was negative. Urine was normal.

Operation: By Dr. C. N. Dowd, February 9, 1917. Part of the epididymis with some testicular tissue adherent to it and a bit of the vas deferens was removed.

Pathological Examination: (R. H. Path. No. S. A., 1914.) Specimen was an irregular piece of tissue measuring 3×2 cm. On one surface it had a glistening membrane, the other surface was rough. On cross section it presented a pearly white, firm appearance with areas of a soft gelatinous substance at intervals through it.

Microscopic Examination: In many instances the epithelium lining the tubules of the epididymis had grown so as completely to fill up the lumen which was greatly dilated. The cells were large and irregular, with vesicular nuclei, and cloudy cytoplasm, and in many cases showed atypical mitoses. The cells in the central portion of the tubules were in some cases degenerated. Surrounding the tubules between the connective tissue and smooth muscle bundles were a great number of round cells. These epithelial masses did not always have a sharply defined limiting membrane, but in many cases had

irregular indefinite edges. Most of the vessels of the stroma showed subintimal fibrosis. One or two sections showed relatively normal epididymis tubules in close proximity to the altered ones.

Second Operation: Because of the possibility of carcinoma, the rest of the testicle and epididymis and some more of the vas deferens were removed on February 19, 1917 (ten days after the first operation). They were apparently normal, both grossly and microscopically. The patient was discharged from the hospital on March 17, 1917. November 8, 1917, the patient was alive, but local condition could not be ascertained as he lived out of town.

Case III. E. R., widower, clerk, 57, English, admitted to the Presbyterian Hospital, July 10, 1917.

Present Illness: Four weeks before admission, following a heavy meal, the patient was nauseated and had heart-burn and feeling of lump in epigastrium; he took sodium bicarbonate and vomited a large amount of brownish semifluid material. Since then he had not been nauseated nor had he vomited, but the eructations and sensation of lump in the stomach continued. He could eat regularly, but immediately after meals had epigastric pains and burning sensations, which were relieved by sodium bicarbonate. He was very constipated; the stools were gray and hard, and streaked with bright blood. Jaundice was noticed one week after first attack and grew progressively worse. Skin itched extremely. Patient had been losing weight.

Past and Personal History: Had had all the diseases of childhood; malaria twice; Neisser infection once; had always had vague stomach trouble and constipation; injury to scrotum when twelve years old; never knew of any lump in that region. Patient is the father of eight children.

Physical Examination: The liver extended from the fifth rib to from 10 to 11 cm. below the costal margin on deep inspiration. In the right lobe was a non-tender nodular mass. The general appearance was one of extreme jaundice and some cachexia. Inguinal hernia on both sides. In the left scrotum was a hard rounded mass about 2 cm. in diameter and apparently attached to the epididymis.

Blood Examination: White blood cells, 9,800; polynuclear leucocytes, 50 per cent., lymphocytes, 47 per cent., transitionals, 3 per cent. Red blood cells, 4,500,000; hemoglobin, 60 per cent. Wassermann reaction negative. Red blood cells were resistant to hypotonic saline solution.

Gastric contents showed free hydrochloric acid, 26–44; total acidity, 34–60; otherwise negative. The stool was clay colored and showed no occult blood and no amylase.

Operation: By Dr. A. O. Whipple, July 16, 1917. Exploratory celiotomy. Cholecystostomy. Excision of mass from scrotum. Along the portal fissure, extending from the head of the pancreas to the gall bladder, and blocking off the common and cystic duct by pressure from without, were several very hard nodular masses varying from 0.5 to 1.5 cm. The gall bladder was moderately distended. The hepatic flexure of the colon was firmly adherent to the mass of nodules; the rest of the peritoneal cavity was negative. Nodule in scrotum was attached to the lower pole of the epididymis. It was well defined, but not encapsulated. Cross section gave the typical appearance of

a fibroid. Running through the surface of this mass were many blood vessels.

Pathological Examination: The specimen was a hard nodule about 1 cm. in diameter, to which was attached some fatty and areolar tissue. On section, the cut surface had a yellow appearance, with numerous interlacing strands of fibrous tissue running in all directions.

Microscopic Examination: The major portion of the mass was composed of dense masses of smooth muscle separated from one another by loose bands of areolar connective tissue infiltrated with round cells, plasma cells, and occasional collections of lymphoid cells. Around the periphery of the mass, and to a less extent in its interior, were a considerable number of what appeared to be small blood- or lymphatic vessels, the walls of which were in many cases thin and lined with what appeared to be swollen endothelial cells, giving them the appearance of atypical tubules lined with epithelium. About the periphery were, also, dilated thick-walled veins, the thickening being due to both subintimal and intermuscular fibrosis. None of the sections showed any epithelial cells. The distribution of smooth muscle and connective tissue above described is confirmed by a Van Gieson stain.

Course: The patient developed a postoperative pneumonia, became more jaundiced, and died on the fifth day.

Autopsy: The common, hepatic, and cystic ducts were completely occluded by a firm yellowish mass, 4 cm. in thickness, apparently springing from the cystic duct. The common bile duct on the liver side of this was greatly dilated, measuring 1 cm. in diameter. The liver weighed 1,600 gm. The tumor proved to be a typical primary carcinoma of the neck of the gall bladder and cystic duct, forming a scirrhous mass which pressed on the hepatic duct. Below this point the hepatic duct was collapsed and of normal size, whereas above this point it was enormously dilated. It was not, however, invaded by the tumor. No metastases were found in the liver, lymph-nodes, or other organs.

Discussion:

Dr. Moschcowitz: I am sure we have all seen instances where the differential diagnosis between pure inflammation and carcinoma was often difficult. This difficulty is especially common in certain ulcers of the skin where there is considerable dipping in of the epithelium, and in the edges of gastric ulcer, where we often find deformed tubules, hyperplastic epithelium, etc. These cases of Dr. Stout's are especially interesting because the simulation is so close; indeed, in his second case, I think the diagnosis of carcinoma is as justified as that of inflammation.

Dr. Wood: I think that the section does look like a carcinoma. There are mitoses in the cells, and on a "flash" diagnosis I should call it a carcinoma.

FIBROMYXOSARCOMA OF THE STOMACH; CHORIONEPITHELIOMA OF THE PINEAL GLAND IN A MALE

G. L. ROHDENBURG, M.D.

(From the Pathological Laboratories of the German Hospital and Dispensary in the City of New York)

The first of the two cases presented had the usual history of a gastric neoplasm. The specimen is shown because sarcoma of the stomach is infrequent, the most recent summary of recorded cases, by Medina and Egana (1), showing a total of but 180 in the literature. Contrasted with carcinoma of the same viscus this percentage of incidence is striking. Macroscopically, sarcomata of the stomach form rounded, irregularly nodular masses which often infiltrate considerable portions of the organ. All types of sarcoma have been described, the round-cell and lymphoid type predominating. The investigations of Oberst (2) show that the mucosa is often not involved. Clendenning (3) in his review states that metastasis occurs in 40 per cent. of the cases.

During the past five years, resection of considerable portions of the stomach has been performed 105 times at the German Hospital. Of these 105 resections, 79 were for carcinoma, 5 for carcinoma arising from an old ulcer, 18 for simple peptic ulcer, and 1 each for polyp, fibroma, and sarcoma. These figures do not include those cases of gastric neoplasm in which operations other than resection were performed.

In the present case, the gross specimen consisted of a portion of the stomach, weighing 1,120 grams and presenting a large tumor mass on its anterior wall. The specimen measured 22 $\times$ 15 $\times$ 8 cm. Viewed from the outer surface, the tumor appeared as a somewhat lobulated growth, the surface of which was roughened and adherent to a portion of the peritoneum. From the inner surface it appeared as a fungated, ulcerated mass, in the central portion of which was an aperture, 1 cm. in

diameter, which communicated with the cavity of the stomach. The wall of the ulcerated cavity varied in thickness, in some places being less than 1 cm. in diameter, in others several cm. thick. No enlarged lymph-nodes were found.

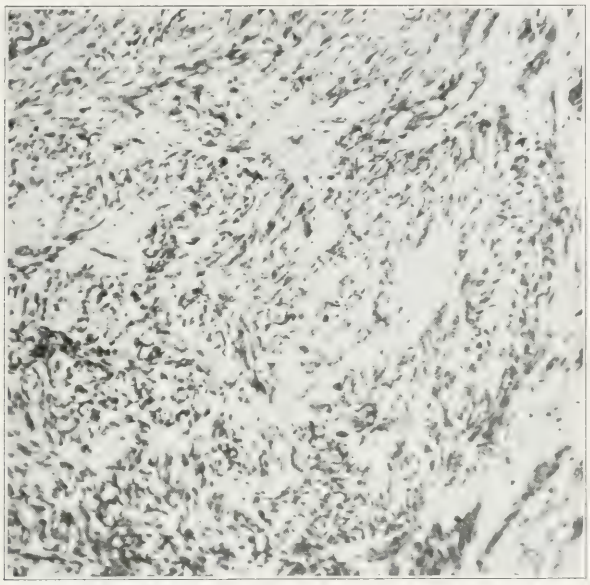

FIG. 1. Fibromyxosarcoma of stomach.

Microscopic examination (Fig. 1) showed the tumor to be composed of irregular bundles and strands of elongated spindle cells of various sizes. In some regions these cells were closely crowded together with a minimum amount of fibrous intercellular substance, while in other areas they were separated by considerable amounts of mucous substance. In the latter regions, the cells were irregular in shape and size, and many had a stellate form. The neoplastic cells for the most part presented rather elongated chromatic nuclei with occasional mitotic figures. The blood-vessels were rather scanty, and those present consisted of thin-wall capillaries, irregularly dispersed among the growing sarcoma cells. Several large areas of degeneration

were present. At the periphery of the tumor there was a fairly distinct capsule of mature connective tissue which sometimes was indefinite in outline, and in most places was more or less invaded by the growing tumor.

The second case to be presented is that of a chorion-epithelioma of the pineal gland in a male. But one other case of a similar tumor type in this locality has been described, a case reported by Askanazy (4), who discovered it at autopsy in a male of 19 years. Askanazy explained the development of the growth by drawing attention to the fact that there were recorded in the literature five cases of teratoma of the pineal gland reported by Guntzeit (5), Neumann (6), Weigert (7), Falkson (8), and Ganderer (9). With these cases as a basis, Askanazy advanced the hypothesis that his case of chorionepithelioma arose from a teratoma. While this, the first case recorded, fulfilled all the requirements of a chorionepithelioma, including the demonstration of stereocilia, still a definite origin from a teratoma could not be shown. The manner of development of a chorionepithelioma from a teratoma is so well known that we shall not discuss the question here.

The clinical history of the case presented here is briefly as follows: A male, thirty years of age, single, a kitchen man, was admitted to the service of Dr. Norbert Stadtmüller, complaining of violent headache for fourteen days, stiff neck, nausea, and sleepiness. Physical examination showed, as the only demonstrable pathological conditions, rigidity of the pupils and a sluggish knee jerk. The patient was under observation for eighteen days, dying in coma. During his stay in the hospital he developed an ocular motor paralysis and some edema of the optic papillæ. His temperature fluctuated between 98.6° F. and 101.4° F. Spinal fluid examination showed an increased cell count and an increased globulin content, the fluid being under pressure. The urine on one occasion contained considerable amounts of albumin and casts, on another occasion sugar, and at still another time was normal. All other examinations gave negative results.

At the autopsy by Dr. Frederick Humphreys the only pathological condition found was in the skull. The testes showed neither gross nor microscopical abnormalities.

Upon opening the skull cap, the dura and pia mater were found to be tense, congested, and edematous, though there was no exudate. The convolutions of the cerebral hemispheres were markedly flattened by pressure, but showed no asymmetrical

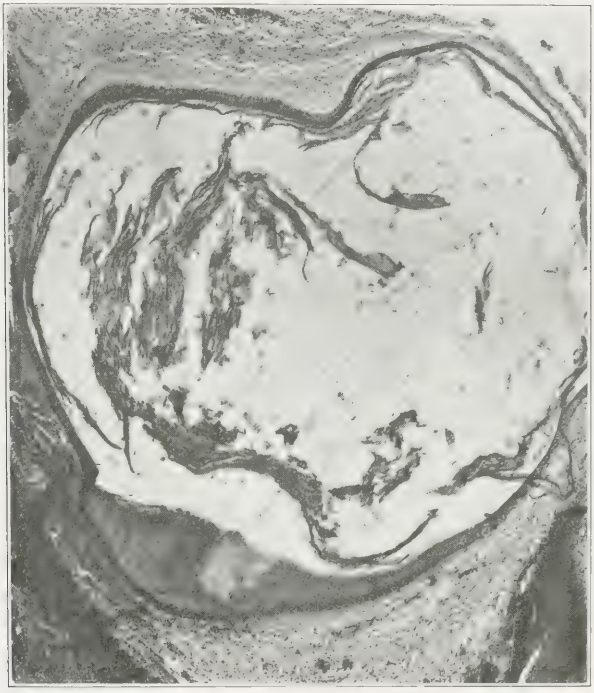

FIG. 2. Squamous epithelium, cartilage, and bone in chorionepithelioma of pineal gland.

bulging. On the upper surface of the brain, in the situation of the pineal gland between the corpora quadrigemina and the superior surface of the cerebellum, was a round ovoid tumor mass measuring $2 \times 1.5 \times 1.5$ cm. in diameter, deep reddish purple in color, and resembling an old blood clot. The attachments of this mass to the pia mater and to the underlying brain substance

were very loose. On section through the tumor it was difficult to determine whether it consisted solely of blood clot or whether there was also organized tissue contained in it. At one point near the periphery of the reddish tumor was a sharply circumscribed, round, white nodule 4 mm. in diameter. This small, white nodule showed a hollow center, the remainder of the walls being thick and white. Running through the reddish material was a fine network of greyish-white threads.

Microscopical examination showed the tumor to consist for the most part of blood clot containing isolated cells or small areas of typical syncytium. The small, white nodule previously described consisted of a circular rim of stratified squamous epithelium, one small portion of the circle containing a nodule of cartilage and early bone formation (Fig. 2). The major portion of the nodule consisted of syncytial cell groups, the cells of the Langerhans layer, also, being present in fair numbers. Stereocilia were demonstrable in scattered areas of the syncytium masses.

The tumor is comparable in its origin therefore with the chorionepitheliomata of the testes which have been recorded, and, finally, it is a proof of the correctness of Askanazy's hypothesis.

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Discussion:

Dr. Moschcowitz: My impression is that sarcoma of the stomach is much more frequent than the figures of Dr. Rohdenburg would indicate. In my own short career I have seen at least six cases, some of which I have reported. Clinically, these cases are distinguished by the fact that they do not show abnormal gastric contents, and that the growths have a marked tendency to be smooth and polypoid in shape, as opposed to typical carcinoma.

THE STANDARDIZATION OF GUINEA-PIG SERUM FOR COMPLEMENT FIXATION TESTS, ESPECIALLY IN REGARD TO THE TITRATION OF THERAPEUTIC IMMUNE MENINGOCOCCUS HORSE SERA

M. A. WILSON

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During the work of standardizing by complement fixation the therapeutic immune meningococcus horse sera, so many difficulties have been encountered in the attempt to determine the accurate antibody content that for the past two years the interest of our department has been largely centered upon that subject.

The tests upon which the standardization of a new serum is based consist of titrations of that serum in comparison with the titer of known sera. In order that the comparison may be true, it is necessary that the reagents used in making the tests shall be as nearly constant in their reactions as possible, leaving as the only variable the unknown serum to be tested. This would be a simple matter, were it not for the fact that the complement is also a variable factor, and attempts to render it constant are not always successful. It is true, then, in this, as in all other complement fixation work, that the value of the tests depends upon the accurate measurement of the dose of complement to be used.

Formerly, this complement dose was measured in regard to its hemolytic strength, an exact hemolytic unit being used with two hemolytic units of the amboceptor. If the fixation and hemolytic units of a given complement were always proportionate, this method for determining the complement dose would be a good one; as a clear-cut hemolytic reaction is quickly and easily obtained when the complement is in good condition. Unfortunately for the convenience of the technician, the hemolytic and fixation strengths of a guinea-pig serum are many times

diverse, and to measure the dose of the complement by its hemolytic unit will not give the accurate titer of the unknown serum. This fact was demonstrated so many times that experiments were undertaken to obtain a method which would give uniform results.

The fundamental idea in our experiments has been to determine the fixation strength of the complement to be used, and to adjust the hemolytic system to meet the requirements of that fixation strength.

After repeated experiments in comparison with the old method, we have found the following technique to give uniform results, and this is the method now in use for all of our standardization tests:

First of all, it is essential that the guinea-pigs be healthy, large, and firm, not fat. The animals are kept for an hour or two without food before killing, and are then bled to death from the carotid arteries, the blood being collected in sterile, neutral Petri dishes. The serum is removed from the clot after standing in the ice-box over night.

After the serum is removed, that from each pig is kept separate until it has been given the following tests:

1. Test for hemolysis with known hemolytic amboceptor.
2. Test for natural antisheep amboceptor.
3. Test for anticomplementary reaction with meningococcus antigen.
4. Test for anticomplementary reaction with meningococcus serum.
5. Test for anticomplementary reaction with heterologous serum.
6. Test for hemolytic reaction with meningococcus antigen, less hemolytic amboceptor.
7. Test for hemolytic reaction with meningococcus serum, less hemolytic amboceptor.
8. Test for fixation with the combination of meningococcus antigen with meningococcus serum.

The importance of making these preliminary tests before pooling the complement from several pigs has been shown in our daily tests of the complement to be used both for meningococcus and gonococcus fixation. Many times in a lot of complement from ten pigs to be pooled, several will not be fixed by the combination of meningococcus antigen and serum, and the same is true with the gonococcus combinations. Therefore, if accurate results are to be obtained with those antigens and sera, the preliminary tests must be made. The most serious objection to this work is the fact that it takes so much time and requires also an extra amount of complement. But, when one considers the amount of material and time wasted when tests are made with complement which has not been accurately tested, the additional expenditure would seem to be justified.

After the results of the preliminary tests are obtained, the sera which have been found to have adequate fixation and hemolytic strength for meningococcus fixation are pooled, and the pooled complement is tested to determine the exact complement dose to be used. This is accomplished by titrating the standard dilution of a known meningococcus serum with two standard units of meningococcus antigen and with varying dilutions of the pooled complement. For the standard dose of complement to be used in testing the unknown sera, we use 0.1 c.c. of that dilution of the complement which gives the standard titer of the known serum in the required fixation time and which gives complete hemolysis of all controls within thirty minutes.

Titrations of Unknown Sera.—Having found the most favorable dose of complement, the unknown sera are titrated with that in the presence of two standard units of the meningococcus antigen. The tests are placed in the water-bath for thirty minutes for fixation of the complement. At the end of the fixation period the indicator, consisting of a 1 per cent. suspension of sheep cells sensitized with two units of hemolysin, is added, and the tests are replaced in the water-bath for one hour. The reactions may be read at the end of the hour, or the tests may be placed in the ice-box over night, and the reactions read from the super-

natant fluid in the morning. If the latter method is used, it is essential to have a color control of each serum, otherwise the reactions may be inaccurately read. The reason for this is that some of the sera have enough color, ranging from a pale lemon to deep orange, to give a tinge of yellow to the supernatant fluid, which, if read in comparison with a serum having no color, would give an inaccurate titer. I am in favor of reading at the end of the hour by the method employed for reading a diagnostic test.

Application of this Method to Diagnostic Tests.—The standardization of complement by this method has not been applied to the examination of patients' sera for diagnostic purposes, except as a matter of experiment, and not enough work has been done upon that phase of the test to justify a statement as to its value there. However, I can say without hesitation that the preliminary tests of the pigs' sera before pooling show a marked difference in their reactions with the gonococcus and tuberculosis antigens. Upon no other antigen and serum combinations has it been tried as yet.

In the work reported here we have included no reactions in which all the controls were not completely hemolyzed, the hemolytic reaction reading being compared to a control of cells hemolyzed by the addition of water. The fixation reaction readings have been controlled by a suspension of cells in saline, thus ruling out, as far as possible, the question of personal equation in reading.

Points of difference of this Method over the Ordinary Laboratory Method.—The only points of difference of this over the ordinary laboratory methods are that we have based our measurement of the complement dose upon its fixability and not upon its hemolytic strength only; and that before pooling the sera from a number of pigs we have tested each one for its fixability with the antigen and serum combination. The hemolytic strength is, of course, taken into account, as is also its anticomplementary reaction with various reagents, as has been shown in our description of preliminary tests of the sera before pooling.

Advantages of this Method.—By making the preliminary tests before pooling, and by finding the exact fixation strength of the pooled complement before proceeding to make the tests of the unknown sera, much time and material are saved, and an accurate titer of the unknown serum is obtained.

The following table shows the variation in titer of one bleeding of meningococcus horse serum when tested with complement standardized by the old and new methods. The standard titer of this bleeding was .0004 c.c.

Number of Guinea-pigs Killed	Number Efficient for Meningococcus Fixation	Hemolytic Unit of Pooled Complement	Fixation Strength of Pooled Complement	Titer of Meningococcus Horse Serum
		Old Method		
10	No test for	.006 c.c.	No test	.0004 c.c.
6	Fixability	.005 c.c.	" "	No complete fixation
10	Before pooling	.007 c.c.	" "	.001 c.c.
6	" "	.008 c.c.	" "	.0008 c.c.
		New Method		
10	4	.005 c.c.	.007 c.c.	.0004 c.c.
6	All	.008 c.c.	.008 c.c.	.0004 c.c.
6	5	.004 c.c.	.005 c.c.	.0004 c.c.
6	3	.004 c.c.	.005 c.c.	.0004 c.c.

Conclusions

1. All guinea-pig serums are not fixable by the combination of meningococcus serum and meningococcus antigen.

2. Each guinea-pig's serum should be tested for fixability before being pooled for meningococcus tests.

PRIMARY PLEXIFORM CARCINOMA OF THE FALLOPIAN TUBE ASSOCIATED WITH TUBERCULOSIS

ELISE S. L'ESPERANCE, M.D.

The comparative rarity of reported cases of primary carcinoma of the Fallopian tube is remarkable. This fact is the more striking when one considers that inflammatory conditions, gonorrheal and tuberculous, of this organ are probably the most frequent form of pelvic inflammation. Orthmann in 1888 described the first true primary carcinoma of the Fallopian tube, and since that time in the entire literature to date there are less than 100 reported cases of undoubted primary carcinoma of the tube. This comparative infrequency may be accounted for in part by the more or less superficial examination given to many cases of pyosalpinx. Spencer in 1916 reported a case operated on for myoma of the uterus; an associated pyosalpinx was removed at the same time. Nine months later the patient returned with a metastatic carcinoma of the inguinal lymph-nodes, and a delayed examination of the supposed purulent salpingitis revealed a fully developed primary carcinoma of the Fallopian tube. Cullen in 1910 reported a similar experience.

The chief question in the histogenesis of this tumor is whether the carcinoma arises from normal surface epithelium or from cell rests in the submucosa or from the atypical proliferation of epithelium associated with chronic inflammatory conditions. Certain writers have apparently observed transformation of normal surface epithelium into tumor tissue and believe that in certain cases this histological picture may be demonstrated. The evidence in favor of this view seems quite insufficient, as apparent transformation may be observed in many actively growing tumor processes.

Lockyer in 1916 emphasized the close relation between salpingitis nodosa and carcinoma and demonstrated precancerous changes in nodular salpingitis which suggest the starting point

of carcinoma. This opened a wide field for speculation in which many have indulged, among them Meyer, Hisli, Grunbaum, and Schultze, who consider salpingitis nodosa as an old tuberculous lesion associated with adenomyoma of the tube with the possibility of later development into carcinoma. Undoubtedly the possibility of the origin of carcinoma from these cell rests should be considered, but the fact that the vast majority of primary carcinomata of the tube involve the mucosa in their earliest stages would rather favor an origin from the latter.

Within the past decade much has been written on the close association between inflammatory hyperplasia, precancerous conditions, and true tumor processes. Bruno Wolff (1897), Le Count, and others, claim that all primary carcinomata of the Fallopian tube arise in organs which are the seat of a chronic salpingitis. The association of chronic salpingitis in all but one or two of the reported cases would appear to confirm this observation. Borst has stated that when carcinoma is combined with an atypical character of the epithelium and heterotopia, one must admit a previous chronic inflammatory process which gives rise to the atypical overgrowth and predisposes to cancer. He considers the term "inflammatory" to cover tuberculous lesions also. More recently v. Franque has described at great length the active proliferation of epithelium associated with tuberculosis of the Fallopian tube and has suggested a close morphological resemblance between tuberculous changes in the tube and true plexiform carcinoma. Lady Barret, during the past year, reported a case of primary plexiform epithelioma of the tube with tuberculosis of both tubes. The natural assumption is that the tuberculous process is primary, and it is possible that an arrest of this process before destruction of tissue occurs may leave the epithelium in such a state of activity that it takes on a carcinomatous character.

The following case may be of some interest as a primary carcinoma of the Fallopian tube associated with tuberculosis. The history of the case is briefly as follows: Mrs. B., aged thirty-five, was admitted to the New York Infirmary on April 22, 1916, with

the history of irregular uterine hemorrhages for the past four weeks. She also complained of considerable leucorrhea. She had lost considerable weight in the past few months. Her family history was negative.

Pelvic examination showed the uterus enlarged, firm, slightly irregular, and fixed. The increased area appeared to be situated between the cervix and the fundus. The cervix was hypertrophied, but not eroded. The external os was filled with reddish material. The tubes and ovaries could not be palpated. A curettage was done for diagnosis and a small portion was also taken from the hypertrophied cervix.

Microscopical examination of the curettings revealed an atypical plexiform carcinoma in a granulomatous stroma with normal uterine glands. The tissue from the cervix showed a chronic endocervicitis. Three days later a hysterectomy and bilateral salpingectomy were done. At the time of the operation there was found on gross inspection a double salpingitis and a myomatous uterus with a wide inflammatory condition in the left broad ligament.

The patient made an uneventful recovery and was discharged from the hospital on May 16 in satisfactory condition. In November, 1917, the patient was still in excellent health.

The pathological examination showed an irregularly enlarged uterus with a small nodular enlargement in the right wall, which on cross-section proved to be an intramural myoma. The cavity of the uterus appeared normal and the endometrium was smooth as from a recent curettage.

Anatomical Description.—The right tube (Fig. 1) was dilated, especially at the abdominal portion, where the fimbriæ were fused in a solid firm mass. This knob-like mass surrounded and was firmly adherent to the ovary. The uterine portion of the tube was narrowed, and the lumen was small. The wall was immensely thickened and was the seat of a diffuse process, involving the entire wall. The mucosa showed irregular papillary projections, which were fused together toward the abdominal portion, completely occluding the lumen. The left

tube was more markedly dilated throughout its entire length, and presented the picture of pyosalpingitis. The abdominal and middle portions were occluded and the lumen was filled with

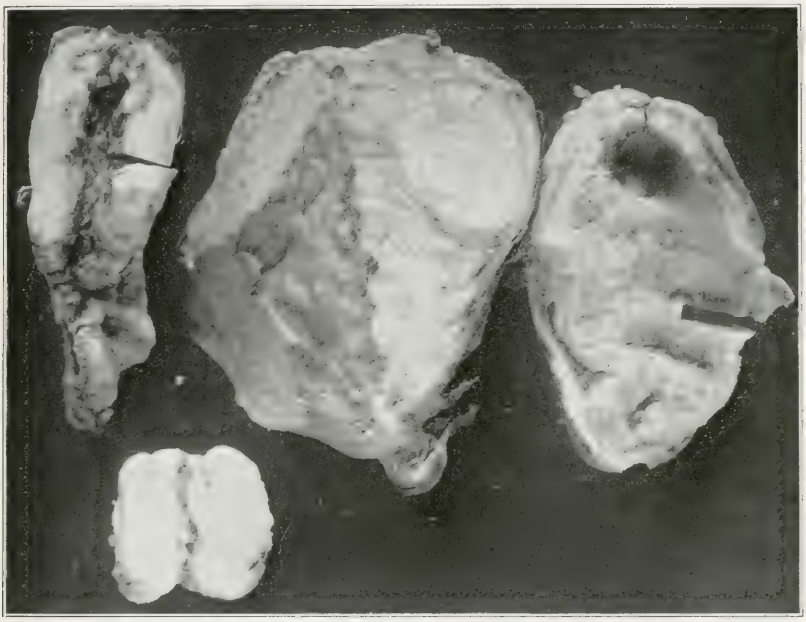

FIG. 1. (1) Right tube; thickened, the seat of a plexiform carcinoma. (2) Left tube: typical tuberculous salpingitis. (3) Uterus: myoma.

necrotic material. The villi were missing, or were fused together, forming a thick velvety surface. The peritoneal surface was roughened and irregular.

Microscopical examination of the left tube (Fig. 2) showed miliary tubercles apparently scattered through a diffuse granulomatous stroma which had almost entirely replaced the normal mucosa of the tube; there was extensive lymphoid infiltration throughout the muscle wall; the lumen contained necrotic material and few pus cells. The right tube was extensively infiltrated with cancer of the type of plexiform carcinoma with areas suggesting epidermoidization (Fig. 3); many necrotic and hy-

perplastic areas resembled tubercles; the muscle coat was deeply infiltrated with cancer, forming nests and strands which extended to the peritoneal surface. The areas of necrosis appeared to develop cysts which had become filled with necrotic material and masses of carcinoma cells. Many of these cyst-

FIG. 2. Tuberculous salpingitis of left tube.

like areas suggested the spaces between former villi, and in certain regions the cancer cells appeared to be continuous with the hyperplastic remnant of the tube epithelium. In certain portions, the arrangement suggested an original papillary character. The great bulk of the tumor, however, was arranged in broad sheets of large polyhedral cells. Many of these masses showed syncytial-like giant cells.

In the study of this case several facts appeared to be of importance in determining the original site of the new growth. First, there was the presence in the uterine curettings of areas of atypical plexiform carcinoma embedded in masses of granulomatous stroma associated with apparently normal uterine glands.

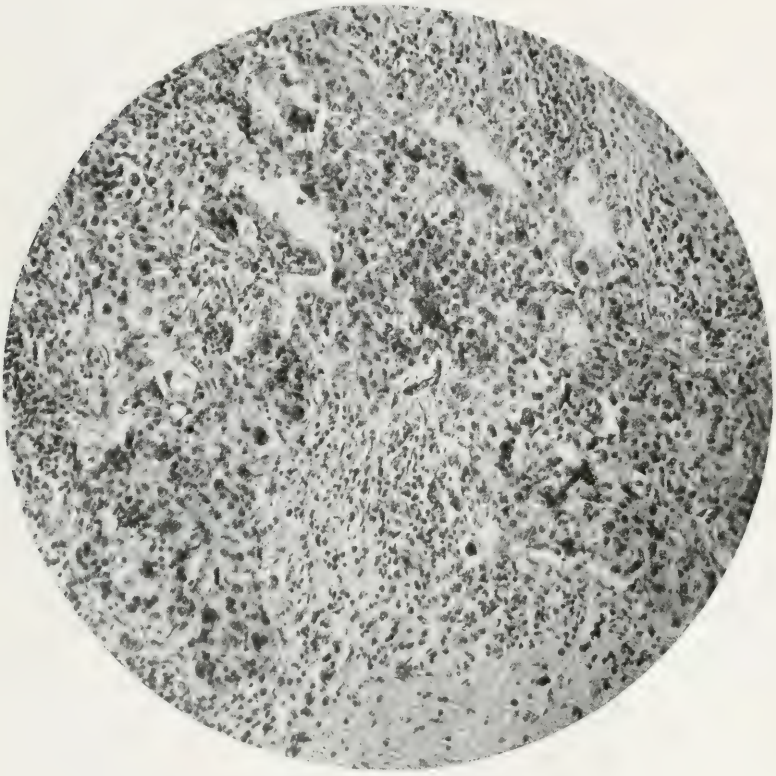

FIG. 3. Plexiform carcinoma in right tube.

These areas suggested an origin from the cervix, but histological examination revealed only a chronic endocervicitis without erosion. The macroscopical examination of the uterine mucosa showed a smooth or very slightly roughened surface, and as the amount of curetted material was very small, it would appear to have been impossible successfully to curette away practically all

trace of tumor tissue from the uterine mucosa. Microscopical sections from many areas showed a denuded uterine mucosa, as one would expect after curettage, with many small, normal, uterine glands. In one area there was a small focus of carci-

Fig. 4. Uterine sinus, showing plug of carcinoma cells.

noma beneath the uterine mucosa. In the sinuses of the uterine musculature there were, however, occasional plugs of carcinoma cells which strongly suggested the method of extension of the tumor to the uterus (Fig. 4). The right Fallopian tube showed an extensive, widely infiltrating plexiform carcinoma involving the greater part of the outer and middle portions. The uterine end appeared small and the mucosa was the seat of a chronic

granulomatous process of the type usually seen in tuberculosis. The left tube was the seat of a typical tuberculous salpingitis; and it is, therefore, justifiable to assume that the granulomatous areas in the right tube were of the same nature.

To summarize this case briefly: we believe that we have a primary carcinoma of the Fallopian tube developing on a tuberculous process with extension of the carcinoma through the vessels into the uterine wall and secondary involvement of a small area of uterine mucosa.

Discussion:

Dr. Ewing: I find this case one of considerable interest in connection with a number of other cases of tuberculosis of the tube which I have seen, where there is an unusual tendency to atypical proliferation of the cells. I have in several instances noted that the appearance of the Fallopian tubal mucosa in tuberculosis was hyperplastic to an unusual degree, and the cells were atypical, with a tendency to diffuse proliferation, and I have often wondered that carcinoma did not develop more frequently. Whether the explanation of the mode of extension in this case is correct or not, I am unable to say. It would be comparable to those types of esophageal carcinoma which travel long distances and reappear in the gastric mucosa, or again, to the carcinomatous lesions of the intestinal tract which appear in the deep lymphatics at a distance, so that such an event taking place in the Fallopian tubes and the uterine mucosa would be no unusual occurrence. Whether the lymphatic or blood supply favor the extension I am unable to say, but I think this one of the most interesting features of the case.

Dr. Moschcowitz: The association of tuberculosis and carcinoma in the same tissues is fairly common. Some years ago I demonstrated before this society a carcinoma of the kidney pelvis associated with a renal tuberculosis; and I have seen carcinoma of the rectum associated with a local tuberculosis a number of times. The interesting point is to determine when the tuberculosis is secondary and when primary. My own impression is that more frequently the tuberculosis is a secondary infection. In certain instances, however, as in Dr. L'Esperance's case and in cases of epithelioma engrafted on a lupus of long standing, the tuberculosis seems to have been primary. If, as Dr. L'Esperance says, salpingitis nodosa is a precancerous stage for carcinoma of the tube, it seems to me that primary carcinoma of the Fallopian tube should be more common in the isthmus, where such lesions are most frequent.

Dr. L'Esperance: The relation that carcinoma of the tube bears to salpingitis nodosa is comparable, I think, to the relation between the adenomyoma of the uterus described by von Recklinghausen and carcinoma. It has been suggested that the same relationship existed between salpingitis nodosa and a true carcinoma of the Fallopian tube. It seems to me, as has

been suggested, that there are so many cases of salpingitis nodosa that it would be a rather peculiar thing to consider the condition as always precancerous. As regards the statement that carcinoma of the isthmus is common, I think that, on the contrary, the outer end of the tube is usually involved. The uterine end of the tube is rather more apt to be uninvolved. At the same time the question of the origin from a tubal adenomyoma, which is thought by many to be associated with salpingitis nodosa, I think ought to be considered.

A SUITABLE MEDIUM FOR THE ISOLATION OF THE MENINGOCOCCUS

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For many years the growth, and particularly the primary growth, of many of the so-called delicate organisms has been supposed to be dependent on the addition to standard media of serum or serous products, although many attempts have been made to devise media which are sterilizable and transparent, and which would take the place of the serum media. It is only in the last few years that certain English observers have pointed the way to a more definite knowledge of the different factors concerned in bacterial growth. Such factors are the presence of easily available protein constituents (as amino acids or otherwise), the proper hydrogen ion concentration, and the presence of certain substances (whose exact nature is unknown) which tend to promote both initial and secondary growth. These last substances I propose to call "Hormones," a term sufficiently indefinite to cover their possible nature. The question of their being true vitamines is probably ruled out by their adsorbability by filter paper. The so-called hormones are taken up by colloidal solutions, such as agar or gelatin, but are readily adsorbed by cotton or filter paper. I can confirm the observations of Cole and Lloyd and Dorothy Lloyd on this point, one passage through filter paper removing at least half of the power to promote initial growth. Since the media (tryp-agar, etc.) pre-

pared by these workers are by no means simple in their composition or method of preparation, it was determined to try various means of securing a medium which would be simple in composition and in the method of preparation, and which, it was hoped, would be efficient enough to take the place, at least in part, of serum media.

After many trials the method given below was adopted. Most of my experimental work has been with media prepared according to the second method, but the first one gives as good growth results, although the final product is not apt to be as transparent. On this medium, the meningococcus has been isolated from spinal fluid in two cases and the gonococcus from urine in three cases. The colonies of both organisms were numerous, were as large or larger than those on serum media, and were microscopically typical; but to the naked eye they were somewhat less succulent and more opaque than usual and showed a distinct tendency in forty-eight to seventy-two hours to differentiate into a rather thick, opaque central portion surrounded by a thin translucent zone.

Early generations of these cultures were transferred to a semisolid (0.5 per cent. agar) hormone agar and remained alive without transfer over a period of ninety-two days, the extent of the observation at present.

I have here as demonstrations plates of various ages of the meningococcus and gonococcus, and also slant agar cultivations. The latter are especially suitable for the production of sufficient material for the manufacture of antigen, since the growth is easily removed and avoids the danger of the introduction of serum from the medium.

Comparative tests against a standard agar made with the same meat and peptone, and having approximately the same titration, show this medium to be at least ten times as efficient as the standard agar in the establishment of primary growth, since ten times as many colonies resulted from the inoculation of the same amount of material.

Curiously, the pneumococcus Types I and II do not grow

especially well unless there is present a small amount of blood, serum, or pus, while Type III shows large, gray typical colonies which, however, tend to flatten out very quickly. Types I and II show a marked tendency to the early formation of ring colonies. Streptococcus colonies are apt to be larger than usual and to show ragged colonies with chain formation on the edge. I am informed by Dr. Torrey that on this medium, when blood plates are employed, hemolysis is more marked, but that the viridans colonies seldom show the green tint and are more apt to be brown in color.

The time is too limited to go into all the different reactions with various organisms, but it is sufficient to state that media prepared in this manner offer many advantages over the standard agars, in simplicity of preparation, in the equipment required, and in the growth ability of the final product. A fluid medium in which 1 or 2 per cent. gelatin is substituted for the agar furnishes a means for the enrichment of meningococcus cultures when made from the throat. On this medium the meningococcus grows as a fine pellicle on the surface, the streptococcus forms long chains which sink to the bottom of the tube, the pneumococcus does not grow well, and the staphylococcus may be held down by the addition of a trace of gentian violet. Smears from the pellicle on the surface show large numbers of Gram-negative cocci; from this pellicle it is very easy to isolate the organism by the plating method.

Discussion:

Dr. Olitsky: May I ask Dr. Huntoon whether the culture of the meningococcus was made from the nasopharynx?

Dr. Huntoon: It was isolated on this medium about two months ago from the spinal fluid.

Dr. Olitsky: Old cultures grow very luxuriantly on plain agar and on the English tryp-agar. Of course, the distinction has to be made between stock cultures and cultures made directly from the spinal fluid or from the nasopharynx. Offhand, it would look as though tryp-agar is a very desirable medium, but used in the field, the results obtained are very disappointing, and positive cultures are less numerous than when laked rabbit blood, or laked human, or laked sheep blood are used. The desirability and the efficacy of any medium would be best tested by finding out whether it would work in field practice, with cultures directly from the nasopharynx. I must say, however, that these colonies of Dr. Huntoon's appear very large.

Dr. Huntoon: All I can say is that I have had no opportunity of making cultures from the nasopharynx. In the cases from which I have isolated the organism the colonies at the end of forty-eight hours were even larger than these. Fishings from the colonies, placed on semisolid medium, lived for six weeks, and at the end of that time they were misplaced, so I do not know how much longer they would have lasted; it was only two months ago that this culture was isolated from the spinal fluid. I had another culture that had been isolated about three months previously, and I was perfectly well aware of the fact that old cultures grow better than fresh ones. I was surprised by the growth on the medium, particularly with the gonococcus, which is harder to keep alive than the meningococcus. I have only laboratory means of testing this medium, but I attempted a comparison between standard agar and this medium. The same amount of meningococcus inoculated on both plates gave ten times as many colonies on my medium, and I believe that there are a number of points in favor of the simpler medium. I do not claim that this can take the place of serum agar; I do not want to give that impression. I think it is about as good as the average serum agar, and I do feel that it can be made up more simply than any agar medium that I have heard of.

Dr. Olitsky: As for the luxuriance of growth on this medium, we had an experience which is of interest. The tryp-agar showed a greater growth on the surface than the sheep serum agar, but on sub-culture, the tryp-agar growth (under the same conditions of moisture) died out in forty-eight hours, while that on the sheep serum was still alive and growing at the end of a week. A distinction must be made between profuse growth and the period of variability with regard to these media. Furthermore, as far as the meningococcus is concerned (I do not know about the gonococcus), it will grow much better and be viable much longer on a laked blood medium.

Dr. Huntoon: I remember back in 1908 when Dr. Elser and I were working on the meningococcus we had some flasks of ascitic broth inoculated with the organism which were placed in the back of the incubator and forgotten. When we cleaned out the incubator we found them, and one of them grew over 285 days. They had been sealed up and left there. I believe that one of the greatest difficulties in preserving the growth of these organisms is the sealing them up so they cannot dry out, and that is one of the principal merits of the semisolid medium, that it retains its moisture.

I should like to add to this paper the fact that the so-called gelatin broth is a fine enriching medium for the meningococcus. I have added a small amount of meningococcus to sputum and inoculated the bubbles formed by shaking the tube. Now these bubbles disintegrate slowly, allowing the meningococcus to float on the surface. At the end of twenty-four hours there will form a streptococcus growth in long chains at the bottom. The meningococcus grows very well at the surface, and the streptococcus at the bottom, so we can make a diagnosis of Gram-negative coccus present by means of the Gram stain from the surface growth.

Dr. Olitsky: This is very interesting, for when saliva is present, the meningococcus will not grow on plate cultures. This is probably due to the bacteria contained in saliva.

TECHNIQUE FOR THE PREPARATION OF "HORMONE" AGAR

F. M. HUNTOON, M.D.

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For Planting and Slant Agar Cultivations: Basis.—Fresh beef heart, trimmed free from fat and tendon and finely chopped. Tap water, 1,000 c.c.; chopped beef heart, 500 grams; 1 whole egg; powdered or granulated agar, 18 grams; peptone, 10 grams; and salt, 5 grams, are mixed in an enamelware water-bath and heated with constant stirring until the color changes to brown (about 68 to 70° C.).

The medium is then titrated by the addition of 4 per cent. sodium hydrate until it reacts slightly alkaline to litmus paper; then 1 c.c. more of 4 per cent. sodium hydrate are added, and the vessel is covered and placed in an Arnold sterilizer or in a water-bath at 100° C. for one hour. It is then removed, the firm clot is separated from the sides, and it is returned for an hour and a half. At the end of this time the vessel is again removed and tipped gently, so that the fluid portion accumulates at one side, and this is removed by means of a large pipette or a siphon, with the avoidance of any fat floating on the surface. The agar is allowed to stand in a cylinder for fifteen minutes, and any fat present is skimmed off. The medium is tubed and sterilized in the usual manner.

Note.—If further clearing is desired, this is accomplished by sedimentation, filtration through glass wool, or centrifugalization.

The Medium at no Time Must Come in Contact with Cloth, Cotton, or Filter Paper. Use.—Plates should dry out in incubator for one hour before inoculation. Slant agar should stand for twenty-four hours before inoculation.

For Preservation of Stock Cultures.—The medium is the same as above, but only 5 grams of agar are employed. Inoculations are made by stab method.

For Fluid Medium.—The medium is the same as above, but 1 to 2 per cent. of gelatin are substituted for the agar.

A second method gives a somewhat clearer end-product. Meat, egg and water are heated with constant stirring to 60° C. and held at that point for five minutes. The meat is strained off through a fine wire sieve and pressed to remove all of the fluid; 1,100 c.c. should be recovered. To the filtrate are added agar, 16 grams; peptone, 10 grams; and salt, 5 grams, and the procedure above is followed.

THE EFFECT OF DISINFECTANTS ON THE ANAEROBIC BACILLI FOUND IN WOUNDS

F. M. HUNTOON, M.D.

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The fact that most of the wounds occurring at the front are infected with a fecal flora, including *B. welchii,* the bacillus of malignant edema, and *B. tetani,* and, also, that gas gangrene may set in very promptly points out the necessity of some means of keeping this infection in check until proper and thorough surgical treatment can be undertaken. Experimental evidence can be adduced to show the importance of the incubation period before any treatment is begun, *B. welchii* in glucose broth cultures growing in a 1 : 40,000 formalin solution when the formalin is added after one hour's incubation, but not growing if formalin in this strength is added immediately after the inoculation. Another point to be considered is the marked influence of foreign bodies, especially rusty metal, on the growth of this organism. The addition of a rusty nail to a plain or glucose broth culture causes a vigorous growth of *B. welchii* in two to three hours, when no anaerobic precautions are taken, the control at this time presenting no visible evidences of growth.

The establishment of a phenol coefficient for any disinfectant, even employing *B. welchii* as a test organism, is useless, since so

many factors enter into the action of disinfectants when employed in wounds. A method of testing devised by Dr. Elser, in which the substances to be tested are superimposed on shake cultures in 1 per cent. agar, has been employed which shows the penetrability of the substance by the zone of inhibition of growth.

A slight modification of this method, in which stab cultures in 0.5 per cent. agar are employed, gives the following results:

Organism	Substance Superimposed	Results
B. welchii	Dakin's solution	No inhibition.
B. welchii	20 per cent. dichloramin-T	No inhibition (first growth at top).
B. welchii	1 per cent. formalin	Slight inhibition.
B. tetani	20 per cent. dichloramin-T	No inhibition.
B. tetani	1 per cent. formalin	Marked inhibition 4 cm.
B. œdematis maligni	20 per cent. dichloramin-T	No inhibition.
B. œdematis maligni	1 per cent. formalin	Marked inhibition 4 cm.

If Dakin's solution is superimposed on a broth culture of *B. welchii*, the first growth to appear at the end of three hours is at the line of demarcation between the two fluids, that is, at the point of greatest concentration. If, however, Dakin's solution is mixed with the culture in the proportion of 1 to 10 immediately after the inoculation, no growth takes place. If dichloramin-T is added, growth is somewhat restrained and gas production is almost absent.

To simulate more closely the conditions found in wounds, the following technique was used: glucose agar in tubes was allowed to solidify around a pipette; when the pipette was withdrawn it left a deep hole in the agar into which a foreign body was introduced, usually a piece of gauze, placed so as to be partially buried but with one portion free in the lumen. This gauze was then infected with *B. welchii* and a small amount of streptococcus or staphylococcus pus and incubated for one hour. It was then treated by various surgical and chemical methods, and re-incubated. Growth of *B. welchii* is indicated by the production of gas, appearing as bubbles in the fluid contained in the lumen, or as gas bubbles in the agar. This method forms a very severe test for any disinfectant, and the results show that Dakin's

solution has little or no effect, and that dichloramin-T has a more marked effect, but by no means shows a complete inhibition. Preliminary washing with 1 per cent. formalin and subsequent treatment with formalin in strengths of from 1 to 1,000 to 1 to 10,000 give the best results of the methods tried.

Further work on this line is under way, and I hope at a later date to be able to present definite results that have been confirmed by clinical observation.

Discussion:

Dr. Ewing: I should like to ask if you have made any attempt to determine how much formalin a wound can stand.

Dr. Huntoon: That is a question which I am unable to answer. That part of the work has been turned over to Dr. Hartwell, and he said he would determine this.

Dr. Moschcowitz: Dr. Brewer introduced formalin about twenty years ago, both as a wash and as a wet dressing, and as far as I can recollect, it had no very irritating effect when used in dilute solutions.

Dr. Huntoon: It does take pretty strong solutions to hold down the *B. aerogenes capsulatus;* it takes at least 1 : 500 dilution.

Dr. Moschcowitz: When you say "1 to 500," is that the dilution of the formalin?

Dr. Huntoon: Yes, formalin, not formaldehyde.

COMPLEMENT FIXATION WITH A SPECIFIC ANTIGEN IN ACUTE POLIOMYELITIS

M. NEUSTAEDTER, M.D., AND E. J. BANZHAF, M.D.

(*From the Research Laboratories, Department of Health, New York City*)

Dr. Neustaedter reported for himself and Dr. Banzhaf the results of their attempts to obtain a specific antigen for complement fixation in poliomyelitis. The method of preparing the antigen was described, and the reactions obtained in a large number of cases were cited. It was concluded that an antigen had been found which is probably specific and fixes complement, and, hence, is of positive diagnostic value in poliomyelitis. This result, the authors stated, was in accord with the production

of immune bodies in a horse injected with poliomyelitic virus digested with trypsin as described.

The paper has been published in full in the *Journal of Infectious Diseases*, 1917, November, xxi, 515.

Discussion:

Dr. Ewing: If I may be permitted to comment on this from the viewpoint of one who is not a serologist: I have always thought that a great deal must depend on the purity of the virus, and I should like to ask what portion of tissue proteins are in the material used as the antigen. Of course one can not get a pure culture.

Dr. Neustaedter: We used a 5 per cent. suspension of brain and cord. This was filtered through a Heim or Berkefeld filter, and to this we added trypsin. Of course we had no means of testing the amount of virus. In nearly all cases we got practically the same results, about 2.5 c.c. of nitrogen from 1 c.c. of suspension.

Dr. Ewing: It is not, then, possible in dealing with these digested proteins that one might get some specific reaction simulating the specific virus reaction? Would it not be well to try some materials from sources entirely removed from poliomyelitis?

Dr. Neustaedter: We have controlled that. We used thromboplastin, which is nothing but a suspension of ox brain in water, and a suspension of normal human brain.

Dr. Ewing: Have you used simple nerve tissue?

Dr. Neustaedter: Yes, but it gave no results at all in this work.

ECTOPIC AND LATENT CHORIONEPITHELIOMA

ELI MOSCHCOWITZ, M.D.

(*From the Pathological Laboratory, Beth Israel Hospital, New York*)

The specimen I shall present bears many points of interest, in relation to both clinical medicine and pathological anatomy. Furthermore, it affords opportunity for wide speculation in regard to the latent period in the genesis of blastomata in general.

The patient was a woman, aged forty-five, who was admitted to the Beth Israel Hospital on May 14, 1917. Six months previous to admission she had begun to expectorate small quantities of blood. There was dyspnea on exertion, which slowly became

worse. Six weeks before admission she began to have pain in the left side of the chest on coughing or on taking a deep breath. This had continued until admission. Patient thought she had lost some weight. Menstruation had begun at thirteen, occurred every twenty-eight days, and lasted seven days. The flow was copious. *Menstruation was regular up to the day of her death.* She had had nine children and one miscarriage. The youngest child was five years old. At no time since the birth of the last child had there been a skipping of a menstrual period, or any other evidence that she might have been pregnant.

Physical examination upon admission revealed all the classical signs of a new growth at the base of the left lung. There was dullness, almost complete absence of breath and voice sounds, and diminished resonance. *X*-ray examination showed a solid shadow occupying this area. There was profuse, bloody expectoration which continued until death. Two weeks after admission signs of a pleural effusion became evident, whereupon aspiration was performed, and a large quantity of bloody fluid was obtained. This effusion also persisted until death. The patient rapidly became emaciated during her stay in the hospital; her hemoglobin, which on admission was 80 per cent., fell to 55 per cent., and the red blood cells dropped from 4,000,000 to 2,900,000. The urine on admission was clear; later it became blood tinged, and this, also, continued until the end. In the last six weeks she had an irregular fever, varying between 100° and 101°, occasionally rising to 103°, but in the last few days her temperature became normal.

At autopsy, both lungs were studded with numberless hemorrhagic tumors, varying in size from that of a pea to that of a walnut, and sharply circumscribed. The tumors were so abundant that the pleural surface was knobby. The whole lower and middle lobe of the left lung was completely solid with tumor, and in the center of the lower lobe was a small, irregularly shaped cavity with necrotic walls, about the size of a walnut. The portions of the lung free from the disease were slaty-green, and showed evidence of compression. The pleural surface was covered with fresh fibrin.

At the upper portion of the right kidney was a tumor about the size of an orange, occupying the entire upper pole. The tumor was very soft, hemorrhagic, partially necrosed, and well defined from the surrounding normal parenchyma. The pelvis of the kidney was involved.

The female generative organs were completely free from tumor. The uterus was normal in size and fibrotic. The mucosa was smooth. The tubes were normal and the ovaries hard and sclerosed. The pelvic tissues were also normal.

This case differs from the conventional type of chorionepithelioma in two particulars: first, in the long latent period following the end of the last pregnancy, namely, five years; and second, because there is no primary tumor anywhere in the genital tract. How are we to explain these two phenomena?

1. The Latent Period

A. Occurrence.—In December, 1910, I reported a chorionepithelioma primary in the broad ligament. The specimen was removed from a woman aged forty-six, who showed signs of pregnancy a year before. She had been curetted in Montreal six months previous to admission; why, was not ascertainable. She had been feeling well until a pain in the side made her seek the advice of a physician, who found a mass in the left fornix. This was removed, but recurrence soon followed, and the patient died with local and lung metastases. Frank reported a case some years ago of chorionepithelioma of the pelvis following fifteen months after the last pregnancy, which terminated in an abortion. Geist also reported a case following three and a half years after pregnancy.

I regarded these four instances within my own knowledge as forming a remarkable sequence of rare cases, and worthy of investigation. In looking up reports of similar cases, I found that what I had thought rare was by no means uncommon. Thus Polano summarized thirty-five reported cases in which there was a long latency period. In thirty of these the uterus was involved; in five the chorionepithelioma was ectopic. Out-

erbridge again summarizes the subject in a recent article, after reporting a case occurring ten years after pregnancy. The longest latent period I have been able to find is that of Polano, ten years. Geist remarks that there is a case recorded as occurring eighteen years after pregnancy. I have been unable to find this report; perhaps it is the case quoted by Reis as reported by Devitsky, in which a chorionepithelioma was found in a woman seventy-two years old.

A number of these reported cases occurred in the menopause, this fact alone proving that a latency period may be considerable.

Of course, it must not be forgotten that in some cases the patient may have been pregnant during the supposed latent period at some time later than the definitely proved conception, because we know that menstruation may occur even during the early stages of pregnancy, and that a subsequent menstruation, even if nearly or apparently regular, may signify the occurrence of abortion. My own feeling is that the history must be considered as an unsafe guide in determining whether conception has occurred or not.

B. How is the latent period to be explained? It is a well-known fact that in every normal pregnancy the uterus is invaded by chorionic epithelium, especially by the syncytial wandering cells. Usually that portion of the uterus adjacent to the decidua serotina is involved, but the cells may be present in any portion of the pregnant uterus at any period of pregnancy, even near the peritoneal surface (Poten). Indeed, even the entire chorionic villus may penetrate into the uterine veins. The chorionic cells persist for some time after delivery, as anyone familiar with the histology of the puerperal uterus can testify. As a rule, they disappear, due in all probability to an autolysin. Sometimes, however, they persist for long periods. The most remarkable instance of this phenomenon is that reported by Ries, who demonstrated chorionic villi in the lumen of a uterine vein eighteen years after the last pregnancy. Certainly the photographs furnished by Ries leave little doubt as to their interpretation. These data, it seems to me, furnish sufficient basis for the explanation of the latency of certain chorionepithelioma.

2. How are we to explain the ectopic nature of the tumor which I have described?

Schmorl, as far back as 1893, found syncytial cells of chorionic origin in the lungs in cases of pregnancy. Lubarsch also found them in the liver in cases of eclampsia. Kassjanow found them in eleven successive cases of pregnancy.

As far as I know, chorionic villi have never been found in the kidney, probably, of course, because the uterine veins do not drain into the systemic circulation, unless there is an open foramen ovale. However, on purely theoretical grounds, with the demonstrable principle of retrograde embolism in mind, there is no reason why chorionic villi may not occasionally lodge in the kidney. I believe, therefore, that this is the logical explanation of my case: that chorionic epithelium was carried to the kidney and remained dormant for five years; that a chorionepithelioma developed therefrom, which gave rise to the metastases in the lungs. This is simpler, for reasons which are so obvious that they do not demand argument, than the explanation that the chorionepithelioma was primary in the lungs.

There is another phase in which the latency of this and similar cases gives rise to speculative interest. Chorionepithelioma is distinguished from all other blastomata in that we can determine the date of the invasion of the offending cells fairly accurately. It makes no difference whether we call it a parasite, a *locus minoris resistentiæ,* or otherwise. At all events, the possibility that such cells may acquire malignant properties at so long a period after they have been implanted in the human organism makes us suspect that probably the same period of incubation, if one may so call it, occurs in the other species of cancer. This may be, in fact, it probably is, one of the reasons why the problem of cancer etiology is such an elusive one.

This latent period also helps to explain the not uncommon observations of the late recurrence of cancer, whether in the lymphatic nodes or otherwise. I believe most surgeons can testify that they have seen cases of recurrences in the lymph-nodes occurring after the classical three years. Dr. F. C. Wood,

at this Society, mentioned a case of recurrence in the cervical lymph-nodes thirteen years after removal of the breast.

Of course, in cases of ectopic chorionepithelioma the possibility of origin from a teratoma must be kept in mind. Only recently, at the last meeting of the New York Pathological Society, a case of chorionepithelioma arising from a teratoma of the pineal gland was reported. A teratomatous origin can as a rule be excluded unless we find some remnant of teratoma included somewhere in the tumor.

The points of interest, therefore, that I desire to bring out in this case are the following:

1. That a chorionepithelioma of the kidney and the lung may be present without a primary tumor in the genital tract.

2. That chorionepithelioma may occur many years after the cessation of conception.

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Discussion:

Dr. Schwartz: It may be interesting to Dr. Moschcowitz to know that the case mentioned by him as one of chorionepithelioma occurring ten years after the menopause was described by McCann.

Dr. Ewing: This case is an unusual example of latent chorioma, in that the tumors are limited to the kidney and lungs. Assuming that the history is reliable, it is necessary to assume that normal villi were deported from the uterus during the last gestation several years ago, and that they lodged in the kidney and recently developed into a malignant tumor. This assumption has often been made before, but it has always seemed to me to be without satisfactory foundation. We cannot prove that it occurs, by logic, but must have some definite observation that normal villi are deported and produce tumors. Portions of villi have been found in lung and pelvic veins,

but never in the kidney. There is another possibility, namely, that this is a teratomatous chorioma developing from an aberrant sex cell, or aberrant ovarian rest. Aberrant sex cells in the lower animals have been found scattered along the vertebral column, and in man there is a series of thoracic and abdominal teratomata probably derived from such cells. Neither of these explanations seems very acceptable for the present case.

Dr. Moschcowitz: There is another possibility, that the patient might have had an abortion in the last five years. The mere fact that she menstruated regularly during this time would not exclude the possibility that there was an abortion.

Dr. Ewing: I think that the histories of these patients cannot be relied on.

Dr. Moschcowitz: The teratomatous explanation occurred to me, but as not the slightest amount of teratomatous tissue was present, I excluded such an explanation.

Dr. Cary: In regard to the latency of tumors, I think it is worthy of note that transplantable tumors in animals remain latent for eight months, and after this time begin to grow, and then grow very rapidly.

Dr. Ewing: I had another interesting case of latency at the Memorial Hospital. The patient was a young man of thirty-four, who had a hemorrhagic tumor at the head of the humerus. He had only one eye; the other one had been removed twenty years before. The structure of the tumor at the head of the humerus was large-cell, and I think so far as histological diagnosis could go, it was a melanoma of the choroid.

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A TUMOR OF THE ULNAR NERVE

A. P. STOUT, M.D.

Recently there has been a great deal of interest evinced in tumors arising from the various derivatives of the neural ectoderm. Marchand,[1] Wright,[2] Martius[3] and a number of other observers have described tumors arising from the neurocytes or neuroblasts of the sympathetic and cerebrospinal system; and these tumors are now very generally known under the names malignant neuroblastoma, neurocytoma, ganglioneuroma and chromaffin tumors.[4, 5, 6]. All these cells are the more or less undifferentiated forerunners of the true nerve cells of the sympathetic and cerebrospinal system.

The nerve cells, however, are not the only derivatives of the neuroectoderm. Certain of the smaller cells of the neural crest develop into the so-called "indifferent cells" or "supporting cells" and these in turn form in the brain and cord the neuroglia and ependyma cells; in the spinal ganglia, the amphicytes, and in the peripheral nerves, the lemmocytes which develop into the neurilemma or sheath of Schwann cells.

The origin of the neurilemma cells is still a matter of debate, since, according to one view, they are of mesodermal origin. The majority of neural anatomists, however, believe that certain of the smaller cells in the neural crest instead of becoming nerve cells wander out along the nerve fibers and become the neurilemma cells. This view is strongly supported by the work of Harrison,[7] who found that in certain types the removal of the neural crest resulted in the formation of efferent nerves without neurilemma cells. If this view is accepted, the neurilemma cells must be considered strictly comparable to the neuroglia cells of the neural tube.

The gliomata, or tumors arising from neuroglia cells, are found only in the brain and cord, along the course of certain cerebral nerves, and in the retina. In certain of the retinal gliomata there occurs a concentric grouping of cells about a central

lumen or cavity which is accentuated by a circular membrane which lies at a short distance internal to the innermost layer of tumor cells. Flexner[8] and Wintersteiner[9] believed that these so-called "rosettes" represented cells from the layer of rods and cones, and that the tumor should be called a neuroepithelioma. This view is still supported by some, notably MacCallum[10] and Mallory.[11] Pusey,[12] however, used Mallory's stain and demonstrated that glia fibrils extended from the cells surrounding the rosette to the edge of the lumen forming there a distinct circular ring and sometimes passing beyond this into the lumen. An appearance somewhat similar to this is found in the ependymal gliomata when the ependymal cells form pseudoglandular structures which Mallory[11] has shown to be formed of ependymal cells.

In passing it may be noted that these rosettes differ from those described as characteristic of the neuroblastomata, in that the latter are small concentric aggregations of cells separated from surrounding cell groups by a considerable amount of fibrillar ground substance. The fibers of this ground substance and the fibers forming the center of the rosette take a stain which is characteristic neither of collagen nor of neuroglia. Küster[13] described two tumors of the adrenal medulla with rosettes in them which he and Ribbert believed were similar to the gliomata of the brain and they called the tumors gliomata. These rosettes, however, are, as his drawings show, similar to the formative sympathetic cell clusters and Wiesel[14] recognized this fact. Since that time, authors generally have considered Küster's cases as malignant neuroblastomata. It is hard to imagine how neuroglia could be found in the adrenal medulla.

If the literature dealing with the tumors of the Gasserian ganglian is considered, it appears that Marchand,[1] in discussing the possible sources of origin for his tumor, suggested that it might have come from the cells forming the sheath of Schwann, but finally decided that it was a neurocytoma, or tumor arising from the undifferentiated cells of the central nervous system. Sachs,[15] Spiller,[16] and Dercum, Keen, and Spiller[17] have re-

ported cases which they believed resembled Marchand's cases. Sachs has some good photomicrographs which show that the tumor is composed of solid strands of epithelial-like cells separated by thick strands of connective tissue. Here and there are rounded holes among the tumor cells with a distinct membrane about them. On this account Sachs thought the tumor ought to be called an endothelioma. It is interesting to note that this case and that of Dercum, Keen, and Spiller were malignant tumors metastasizing in the cervical lymph-nodes. None of these investigators used the Mallory stain.

Turning now to tumors arising in the peripheral nerves, one is impressed with the fact that the literature is very scanty, but the majority of authors consulted seem to agree that whether single or multiple, malignant or benign, they are of mesodermal origin coming from the epineural or perineural tissue. Some of them have in addition an overgrowth of nerve fibers.[18, 19, 20] In 1908, on the other hand, Verocay[21] described a case with multiple tumors of the nervous system which showed in many of the nodules in the peripheral nerves what he believed to be a marked growth of cells of the sheath of Schwann. He, thus, is the first author to suggest that the neurilemma cells may be the source of tumors of the peripheral nerves. This view is looked upon with favor by Borst.[22]

In this connection the result of sectioning peripheral nerves is of great interest. Tinel,[23] in a book on Nerve Wounds, which has just appeared, makes the following statement: If a peripheral nerve be sectioned there appears on the central end the classical amputation neuroma. This is composed of tangled nerve fibrils embedded in fibrous tissue which is sprinkled with numerous cells resulting from the proliferation of the cells of the sheath of Schwann. The peripheral end presents a swelling which is composed solely of the excessive proliferation of the cells of the sheath of Schwann. This Nageotte calls a false neuroma or glioma. It is of course not a true neoplasm but a hyperplasia, and is comparable to that which occurs in hyperplasia of scar tissue or false keloid.

The tumor under consideration started nine months before operation as a swelling on the anterior aspect of the right forearm of a male Italian laborer, forty-two years old, who was otherwise apparently well. One and one half months after its appearance he noticed a mass in the axilla on the same side. There was only partial interference with the function of the ulnar nerve noted.

Interscapulo-thoracic amputation was performed by Dr. A. V. S. Lambert, on November 20, 1917. Examination of the arm showed a fusiform tumor occupying the course of the ulnar nerve, apparently starting within its sheath at that point in the palm of the hand where the nerve splits up into its terminal branches, and extending to a point just below the internal epicondyle of the humerus. The tumor was circumscribed and was dissected away from the surrounding muscles only with difficulty. At its thickest point it was adherent to the skin, which was ulcerated. In the axilla was one large mass of the same appearance and consistency as the nerve tumor, measuring $15 \times 14 \times 0.5$ cm., through which passed the great axillary vessels and nerves, some of the intercostal nerves, and the long thoracic nerve. Adjacent to this were numerous rounded nodules from 1 to 2 cm. in diameter which appeared, some in the surrounding muscles, one, at least, in the course of the long thoracic nerve; and others were found at operation in the supraclavicular fossa. These masses have three possible explanations: (1) they may be metastases in lymph glands: (2) they may be direct extensions along the nerve trunk; (3) they may be independent tumors, since tumors of the peripheral nerves are often multiple. The material has not yet been sufficiently studied to determine which is the correct hypothesis. Personally I believe them to be metastases.

The microscopic morphology of the tumor is everywhere the same and is distinctive. There are thick masses and strands of rather small rounded cells, many of which show mitoses, separated by trabeculæ of connective tissue. Scattered through the tumor cells at irregular intervals are many rosettes which closely resemble those described as being found in the retinal gliomata.

These are small circular holes surrounded by a sharply outlined membrane which is red with a Mallory stain. Passing from the membrane between the surrounding cells like the rays of a star are many fine fibrils which in some instances pass over the membrane and are seen in the lumen of the rosette.[1] These are also red with a Mallory stain. The fact that these fibers stain red with a Mallory stain of course does not *prove* them to be akin to neuroglia, since Mallory has shown that not only neuroglia but also fibroglia, myoglia, and fibrin stain red as well.

The close resemblance between this tumor and some of those described as arising from the Gasserian ganglion must also now be apparent. Both are malignant, metastasizing at a distance, and in their microscopic appearance they have many points in common. It is of course possible that this tumor, like Marchand's, may have arisen from neurocytes, since ganglion cells have been found in the course of some of the peripheral nerves.[24, 25] But this is an exceedingly rare occurrence and it is much easier to believe that it arose from neurilemma cells which are constantly present in all efferent peripheral nerves. It is also possible that Marchand may have been nearer the truth in his discarded hypothesis, that his tumor arose from the cells of the sheath of Schwann.

In conclusion, it may be said that the tumor under consideration is a malignant neoplasm. Grossly it resembles the sarcomata, and microscopically and tinctorially it has some points in resemblance to the retinal gliomata and to some of the Gasserian ganglion tumors. Since the neurilemma of the peripheral cells is probably homologous to the neuroglia of the central nervous system, it is suggested that this tumor may be a neoplasm arising from neurilemma cells or lemmocytes.

I wish here to thank Dr. A. V. S. Lambert for his permission to report this case.

Case History

J. T.; 42 years; male; Italian; laborer; married; admitted to the Presbyterian Hospital November 15, 1917.

Chief complaint; lump in right arm. Present history: Nine months ago first noticed a numbness of the anterior surface of the little finger. Soon

after appeared a swelling of the anterior surface of the forearm which was hard and not tender. One and one half months later a lump appeared in the right axilla which was at times very painful. Two months before admission, a cut was made into the mass in the forearm. After this the masses in the forearm and axilla increased rapidly in size and the pain grew very great.

Past and Family History: Neisser infection, twenty-one years ago. Wife had seven miscarriages. Otherwise negative.

Physical Examination: On the volar surface of the right forearm there is a mass which extends from just below the bend of the elbow to just above the wrist. At the lower end it is raised above the skin about 2 cm. and its surface is ulcerated. The mass is firm, slightly tender, nodular, and seems to be attached to the muscle but not to the bone, as it moves freely from side

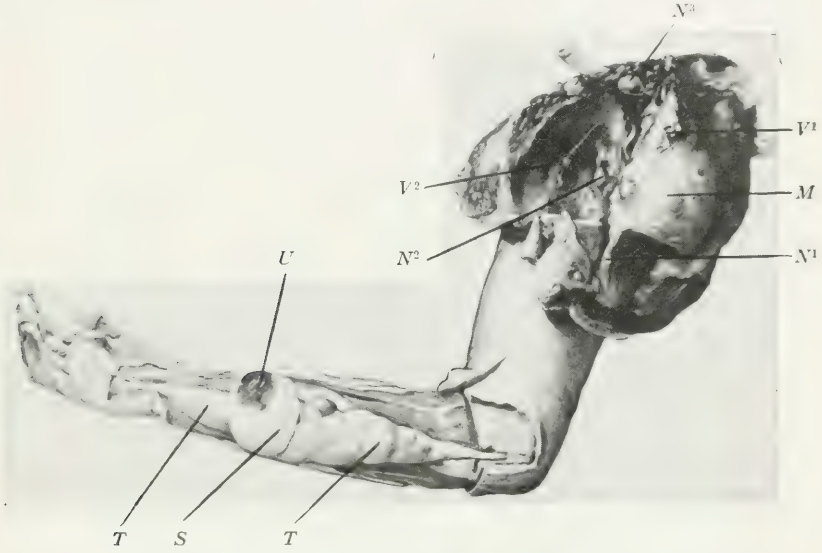

FIG. 1. Photograph of sketch of dissected upper extremity. *T T*, tumor occupying course of ulnar nerve; *U*, ulcerated surface of tumor; *S*, area of skin infiltrated by tumor tissue; *M*, large axillary mass dissected and reflected back from its bed; V^1, great vessels and nerves passing through top of mass (cut and reflected with mass); V^2, the peripheral ends of great vessels and nerves at the bottom of the cavity from which the mass *M* was reflected; N^1, nodule in course of long thoracic nerve; N^2, nodule in substance of subscapularis muscle; N^3, another axillary nodule.

to side. The intrinsic hand muscles show considerable atrophy. In the axilla is a mass, three by four inches, with irregular surface, not tender, and freely movable from side to side. Above the clavicle can be felt some three or four small masses from 0.5 to 1.5 cm. in diameter. In the epitrochlear region can be felt other nodes. Sensation over the ulnar distribution is diminished over the right side.

The lungs show bronchovesicular breathing symmetrically over both chests. No râles. Physical signs were otherwise negative.

Wassermann reaction was negative.

Operation: November 20, 1917, by Dr. A. V. S. Lambert. Interscapulothoracic amputation of right upper extremity.

Post-operative Course: The wound healed promptly and the patient was discharged December 7, 1917, seventeen days after operation, at which time

FIG. 2. Hematoxylin and eosin stain. Medium magnification microphotograph of typical field of tumor. There are many rosettes and trabeculæ of connective tissue. (*R*, rosette; *C*, connective tissue trabeculæ.) Only the most prominent ones are indicated.

there was felt an indefinite mass in the neck apparently above the nerve stumps of the brachial plexus. This was thought to be a recurrence.

P. H. Surgical Path. No. 20,464.

Gross Specimen: Specimen consists of a right arm, including the shoulder joint, scapula, and half of the clavicle (Fig. 1). Presenting on the internal

anterior aspect of the forearm is a large nodular swelling adherent to the skin, on the highest point of which is a recent operative wound surrounded by an area of ulceration. On dissecting the forearm, it is seen that this mass is part of a fusiform, nodular, firm, white tumor mass which starts apparently within the sheath of the ulnar nerve just below the internal epicondyle of the humerus, grows gradually thicker until it is 6.5 cm. in diameter at the junc-

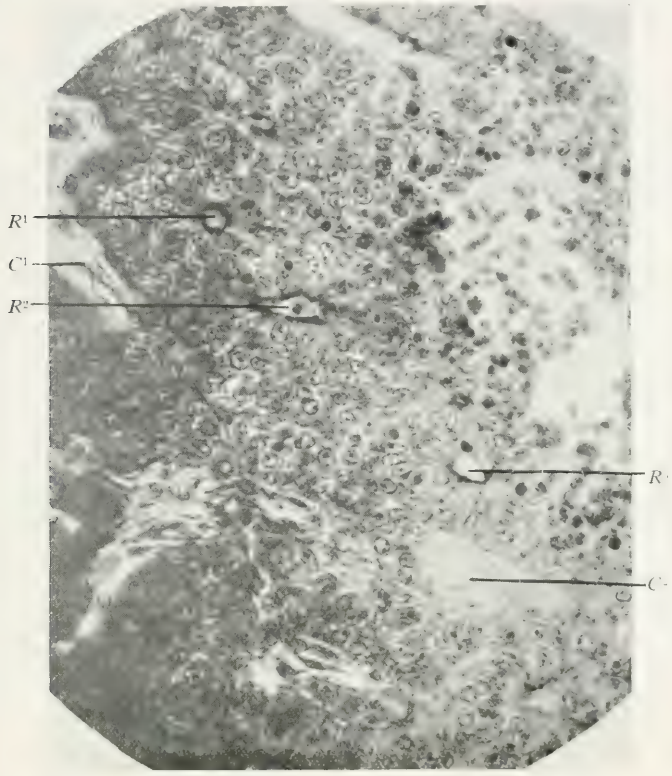

FIG. 3. Mallory stain. High power microphotograph of 3 rosettes, R^1, R^2, R^3, showing deeply stained membrane or rim from which radiates the fine fibrils between the surrounding cells. C^1 and C^2 are trabeculæ of connective tissue.

tion of the lower and middle thirds of the forearm, then rapidly diminishes in diameter until it ends as it began within the sheath of the ulnar nerve just below the annular ligament of the hand. It does not come into contact with bone at any point, but lies in front of and internal to the radius, ulna, and interosseous ligament.

In the axilla extending from the subclavian artery downward for a dis-

tance of 15 cm. along the lateral thoracic wall is a firm, white, nodular mass adherent to the surrounding structures and measuring 5 cm. at its thickest points. All the great vessels and nerves of the axilla and many of the intercostal nerves and vessels and the long thoracic nerve pass through it. The long thoracic nerve after it leaves the mass and splits to pass into the muscle, has a nodule apparently of tumor tissue about 1 cm. in diameter, either attached to or incorporated within the nerve sheath. In close proximity to

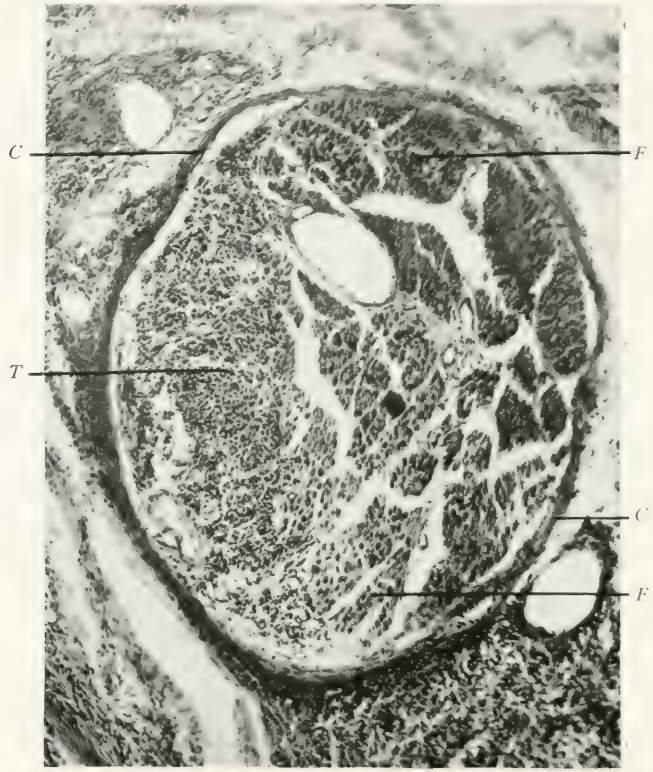

FIG. 4. Photomicrograph of section of tumor taken just below internal epicondyle of humerus. A bundle of nerve fibers in its connective tissue capsule (*c*) is shown with a mass of tumor cells (*t*) sharing the space and in intimate relationship with the bundles of nerve fibrils (*f*). Note large size and hyperplasia of the neurilemma cells.

the large mass are a number of small nodules from 1 to 2 cm. in diameter, some of which are superficial to it; others are deep and buried in the substance of the subscapularis muscle; while still others are found above it both in the axillary fat and in the supraclavicular fossa.

Microscopic Examination: Sections from the mass in the ulnar nerve and the axillary mass show the following architecture: The tumor cells are small, rounded, and of relatively uniform size; the nucleus is relatively large and takes a deep stain with hematoxylin (Fig. 2). There are numerous mitotic figures. The cells are massed, with an alveolar structure separated by thick trabeculæ of connective tissue bearing the blood vessels. Among the tumor cells are many small rounded spaces, some empty and others apparently filled with delicate tangled fibrils. With Mallory's aniline blue-orange G stain, these holes are sharply outlined by a deep red staining membrane from which numerous fine fibrils pass out in a radiating manner like the rays of a star between the surrounding tumor cells (Fig. 3); some of these at least seem to pass inwards into the lumen. With Van Gieson's nerve stain, these fibrils are not nearly as sharply defined. They have a tint which is almost like the red of the collagen fibrils but with a suggestion of orange in it. The tumor cells surround and are separated from the membrane by a space about the width of the nucleus which is filled up with these fibrils. A section taken from the tumor at a point just below the bend of the elbow where it measures only 1.5 cm. in thickness shows about the periphery of the mass of tumor cells some bundles of nerve fibers (Fig. 4). The neurilemma nuclei appear unusually prominent, and within one fasciculus there is seen a strand of tumor tissue in intimate relationship with the nerve fibers. Another fasciculus appears flattened out and compressed.

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CARCINOMATOUS TRANSFORMATION OF A LINGUAL GOITER

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Lingual goiter is a genuine aberrant growth developing in the embryonal remains of an incompletely obliterated thyroglossal duct. It thus differs from the false aberrant goiter, which denotes a process of thyroid tissue secondarily separated from the rest of the gland, but still maintaining at least a connective-tissue connection with the main gland. Although of all the genuine aberrant goiters, namely, the prelaryngeal, suprahyoid and lingual, the last is the most common, there are reported in the literature to date less than one hundred cases.

It is likely that a good many of these aberrant thyroids are never observed because attention is directed to them only when they become a surgical problem. Furthermore, if we consider the embryological factors which make this condition possible, it is probable that lingual thyroids in a quiescent state are more prevalent than is generally believed. It is interesting to note that the existence of aberrant thyroids is frequent in animals, where they can practically always be found in the form of numerous minute accessory nodules of thyroid tissue scattered through the neck, around the thymus or even inside the pericardium. This accounts for the occasional difficulty in demonstrating in animals the effects of the loss of the gland by operative extirpation.

From examination of cases of lingual goiter already published, it is found that the usual site of the tumor is in the median

line (a point of diagnostic importance) in relation to the foramen cæcum. Rarely the thyroid nest may occur along the border of the base of the tongue. The consistence of the mass is tense and elastic. The mass may vary greatly in size, and the surface is generally covered with smooth mucous membrane; because of the extreme vascularity the color is generally dark red or purple. The exposed location of so vascular a growth renders it liable to hemorrhage as a result of trauma.

Wood mentions that, in addition to the pathological interest, these misplaced gland masses are of surgical importance in that in about one tenth of the cases the thyroid tissue in the tongue is the only example of that glandular structure in the body, hence the occasional development of myxedema in the patient in whom extirpation of the tongue is performed.

Another interesting clinical feature attached to these tumors is that they occur almost exclusively in women. They develop in the same manner as true goiter, generally between childhood and the forty-fifth year of life, but may be congenital, and may occur also in old age. They are particularly apt to develop after atrophy of the thyroid or in association with early goiter attended by functional disturbances in the thyroid, or after operative removal of the gland.

The particular interest that attaches to the case I wish to report is centered in the facts that we have such a gland nest in a man who showed no evidence of it until after reaching the age of seventy. The rare extreme lateral location in the tongue, and the apparent malignant transformation, are not mentioned in the literature pertaining to this subject, in spite of the not infrequent malignant neoplasms of the thyroid gland in its normal location.

Case Report.—This case was in the service of Dr. T. A. Smith, whose permission I have to use the clinical record. The patient, a cabinet maker, aged 74 years, was admitted to Bellevue Hospital complaining of enlargement of the tongue and difficulty in swallowing and talking. The patient stated that three years ago he noticed for the first time a small nodule on the left side of the tongue that became progressively larger until it began to interfere with talking, chewing and swallowing. In the course

of two years, he lost about 70 pounds. Physical examination showed an emaciated old man. The tongue was markedly coated and swollen and disclosed on the left side a lemon sized tumor, non-fluctuating, and firmly attached to the upper layers of the tongue. Examination of the neck revealed enlarged, hard submaxillary glands which were attached to the deep structures; the right side disclosed an enlarged lymph node. The lingual tumor was enucleated, and the patient died two days later of bronchopneumonia.

The specimen as received in the laboratory consisted of an irregularly spherical mass, about 5 cm. in diameter, and covered for about two fifths of its surface area by the superficial layers of the tongue. The mass was well enclosed in a thin, connective tissue capsule and the consistency was somewhat boggy. On section, the greater portion of the growth was composed of irregularly lobulated, pale tissue bearing a close resemblance to the malignant adenomata of the thyroid gland. The entire mass was solid, and showed scattered areas of hemorrhage, but there was no fluid or necrosis.

Microscopic examination revealed that the growth was composed largely of acini or vesicles which varied greatly in size and configuration, and were supported by a scanty, moderately vascular connective tissue framework. In places the lining epithelium was cuboidal or low columnar, and in still other places high, arranged in single or double layers and in some vesicles branching into papillary projections. The basement membrane in some areas was not intact, and there were definite indications of carcinomatous transformation. Some of the alveoli contained hemorrhagic exudate, others a pinkish staining homogeneous material corresponding to colloid.

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THROMBOPHLEBITIS OF THE HEPATIC VEINS WITH ACUTE MESENTERIC THROMBOSIS

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The patient from whom this specimen was removed at autopsy was admitted on December 27, 1917, to the medical ward of Dr. Morris Manges; and I am indebted to Dr. Manges for the privilege of presenting the case. The patient was 48 years old, a female, who had been suffering for one year from headache and swelling of the legs and feet, for several months from a cough, and for three weeks from abdominal swelling and cramps. On physical examination the patient was found acutely ill. She was very obese, and showed marked cyanosis of the skin, especially of the face. There were many small hemorrhages in the skin of the lower extremities. Both liver and spleen were palpable and there were signs of free fluid in the abdomen. The lower extremities also showed inflamed varicose veins. The blood count showed the following: hemoglobin, 120 per cent.; red blood-cells, 8,400,000; white blood-cells, 12,000, with 78 per cent. polymorphonuclear leucocytes. The blood Wassermann was negative. The urine showed only a trace of albumin. On December 31, the patient complained of severe cramp-like pains. On January 1, a phlebotomy was performed, and sixteen ounces of blood were removed. After this the patient gradually became worse, and ceased breathing on January 2.

At autopsy, the *heart* and *lungs* were practically normal. The peritoneal cavity contained five liters of hemorrhagic fluid.

Liver was slightly smaller than normal. The free edge of the left lobe was very thin and atrophic in appearance, and its surface was ribbed with fibrous streaks. The surface was smooth and glistening, dark reddish brown in color, and slightly icteric. On section, the central veins stood out prominently and a moderate grade of central passive congestion was present. Near the center of the liver was a large, irregular, pale, anemic-looking

area. The intrahepatic branches of the portal vein were filled with blood-clot which had the appearance of a thrombus formed just ante mortem. This clot was dark red at the center and pale at the periphery and not adherent. The intrahepatic branches of the hepatic veins stood out prominently, their walls being markedly thickened, and they were filled with thrombus, slightly older in appearance than that in the portal vein, also not adherent. The orifice of the hepatic vein into the inferior vena cava presented a very remarkable appearance. These orifices were practically completely closed, some of them with very small openings in them. The edge of these openings was smooth and rounded. Over one of the orifices there was a slightly raised area of densely adherent thrombus material, with smooth glistening surface, apparently covered by endothelium. Similar smaller areas of thrombus were present at the orifices of several of the other veins. The portal vein and its tributaries were distended with non-adherent blood-clot which had the appearance of thrombus formed just ante mortem. This was present in all of the tributaries of the portal vein, including the splenic. The wall of the gall bladder was thickened; otherwise normal.

Esophagus was normal.

Stomach and Intestines.—The stomach was normal in size, and its serous surface was normal. The cavity contained blood stained mucus, and its mucosa was hemorrhagic.

Practically the entire small intestine was hemorrhagic in appearance, almost black in places, showing an early stage of gangrene. The transverse colon presented a similar appearance. The greater omentum was much thickened and hemorrhagic in appearance and its veins were filled with thrombi similar to those described above. The mucosa of the small intestine was hemorrhagic in appearance, and that of the lower portion of the ileum was greenish in color, velvety in appearance, and very friable (necrotic). The mucosa of the entire intestine was also hemorrhagic in appearance. One diverticulum was present in the descending colon. This diverticulum was small and showed no inflammatory process about it. Mesentery of the small intestine

was much thickened and hemorrhagic. Mesoappendix also was thickened, and there were great numbers of dense fibrous adhesions, binding one portion of the mesosigmoid to the other, and causing much twisting of the sigmoid. Similar dense fibrous bands were found along the entire course of the mesocolon.

Spleen was three times its normal size, and densely adherent to the surrounding parts. It was firm, dark red in color, and the capsule was thickened. On section, cut surface presented a uniform dark appearance and contained no thrombi.

Pancreas was normal.

Kidneys.—Normal in size. The capsules stripped with some difficulty, removing some renal material. The surface of the kidneys was pale. Both kidneys were friable.

Adrenals were normal.

Uterus and adnexæ were normal.

Inferior Vena Cava.—Except at the orifice of the hepatic veins (above mentioned) was normal.

Aorta showed slight atherosclerosis with no calcification and no lesion suggestive of syphilis.

Bone marrow from femur was dark red in color.

Microscopical Examination.—Liver and hepatic veins: the liver showed a moderate grade of central passage congestion. All of the hepatic veins showed a moderate grade of dilatation and many of them contained very recent hyaline thrombi. The walls of the veins were normal. The orifice of one of the occluded hepatic veins was filled with almost completely organized hyaline thrombus. The wall of the vein at this situation showed only a slight degree of thickening, due to an increase of connective tissue. This thickening appeared to be secondary to the thrombosis.

It is of interest to note the relatively mild grade of congestion with this degree of obstruction of the hepatic veins. There is no clear explanation which can be given at this time of the etiology of the lesion of the orifices of the hepatic veins shown in this specimen. The closure of the orifices of the hepatic veins is evidently due to organizing thrombi occurring at these orifices.

Since there is no primary disease of the hepatic veins and since the Wassermann blood test was negative, neither of these can be given as the explanation of the original thrombosis. Whether there was an increase in the blood platelets along with the polycythemia was not determined. Therefore, the influence which the polycythemia might have had on the original thrombosis cannot be determined. There is no specific disease of the hepatic veins such as is found in thromboangiitis obliterans of the extremities. Only one explanation, consequently, can be advanced by me to account for this lesion. The situation where this thrombosis occurred is at a place where the blood-stream is slowest in the entire body. The cough which the patient had for several months before death may at times also have further slowed the blood-stream in the vena cava. The hepatic veins enter the vena cava at right angles. These two conditions are known to be important predisposing factors in thrombus formation. The polycythemia might also have contributed to the change in the blood elements which would be an additional predisposing factor to thrombosis. The combination of these three factors in the obese, middle-aged woman can account for the lesion found, and is the most plausible thing that suggested itself to me. This explanation has been advanced by previous observers to account for this lesion. This theory is in accord with the following conclusion of Aschoff as to the explanation of thrombosis.

"So we see from all these experiments that the slowing of the blood stream and the alteration of the blood elements themselves, especially alterations of the platelets, are the chief factors in the production, not only of static, but of toxic thrombosis, so far as we are concerned with thrombus formation in the flowing blood-stream."

Discussion:

Dr. Larkin: I think this is one of the cases seen now and then in which infection plays a very large rôle. We cannot say what the nature of the infecting organism is, or how the bacteria enter into the circulation, perhaps it is through the lung, as Dr. Thalhimer suggested, or perhaps through some other portal of entry. I am anxious to find out whether there had been any injury to the lower venous plexus in this case, and I have been told that no

such lesion was observed. However, I do not think that this element can be overlooked. We know that infection has a predilection for veins in which the circulation is very slow. The condition of polycythemia is also very interesting. Aschoff's explanation of the condition of thrombosis (platelets) has been used to explain a number of similar cases cited by others. The case here bears out very well one which was described by Brill. My idea of the etiology of this lesion is that there is an injury, bacterial in origin, which has taken a rather slow course, and has closed off the small veins going off from the vena cava, producing lesions of the intestine and liver.

Dr. Moschcowitz: There are a number of points which I should like to comment upon. It seems to me that such a damming up caused by stoppage of the outflow of the blood of the liver into the circulation would have a tremendous effect on the liver. I am entirely at a loss to know why there has not been more engorgement than is evident in this case. Another point is whether it would not be possible to make a clinical complex of cases of this sort. It is a question whether the polycythemia is a cause or the result of the lesion. Certainly on speculative grounds there is no reason why a patient with a thrombosis of the hepatic vein should have a polycythemia. The mechanical explanation was advanced by Pankert who found the lesion in patients who had had whooping-cough. He believed that the cough spasms caused a rupture of the intima of the hepatic vein, which, being repeated, caused a widespread lesion of the intima, and thus gave rise to thrombosis.

Dr. Thalhimer: I think with Dr. Larkin that undoubtedly the closure of these vessels is due to the obliterating process which is started first by the formation of platelet thrombi, followed both by organization and additional thrombus formations. It might be that some low grade bacterial infection might have started this process, either by localizing here at the orifices or the hepatic veins, or by some general toxic effect. It would be a most peculiar place for bacteria to localize and so far as I can see there is no particular reason why they should localize here when there are so many other places which are much more likely to catch them in a mechanical fashion. I also marvel with Dr. Moschcowitz that there was no greater congestion of the liver than that found, and I emphasized that fact when the specimen was being shown. Whether collateral circulation might have taken place we have not as yet worked out, but that will be done. There are a number of hepatic veins which had small orifices, and if all of them were put together a good deal of blood could pass through them, just as a large amount of blood goes through a tight mitral stenosis. One sees cases of mitral stenosis of very high grade, and if the patient's condition is otherwise good, circulation will be maintained through the valve for many years of life. Still I do not feel that we have a very good explanation for the condition.

AORTIC ANEURYSM; LYMPHOSARCOMA OF THE GALL-BLADDER; AN UNUSUAL TYPE OF CARCINOMA OF THE APPENDIX

G. L. ROHDENBURG, M.D.

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The three specimens presented are of rather unusual interest from the pathological standpoint. The first specimen, an aneurysm of the ascending aorta, was obtained from a male of 40 years, who was admitted to the hospital with some of the cardinal signs of the condition. The *x*-ray examination, however, confused the clinicians, the reason for the confusion being the irregular and lobulated appearance of the shadow, a condition which prompted the diagnosis of mediastinal tumor. The *x*-ray diagnosis was favored by a negative specific history, and by the fact that repeated complement fixation tests, even with reinforced antigens, were consistently negative. The patient died shortly after an injection of arsenobenzol with all the evidences of arsenical poisoning. Permission was given to open the chest only, in consequence of which no definite statement as to the cause of death can be made.

The specimen (Fig. 1) showed a general enlargement of the aorta commencing directly above the aortic cusps and ending at the first portion of the arch. From the generally enlarged artery there projected several pouches which were rather remarkable for their size and distribution. Directly above the aortic cusps there was a pouch having an opening 3 cm. in diameter. This pouch completely covered the left auricle, though it did not communicate with it. Directly opposite was another pouch, almost as large, which projected directly forward and which was filled with organized blood-clot. At the upper portion of the ascending aorta were two other pouches, smaller in diameter, but of the same general character. These upper pouches had commenced to cause erosion of the trachea. Microscopical examina-

tion showed the chief change to be in the intima and media. In the latter coat there were extensive areas of degeneration with cholesterin crystal spaces as well as an extensive fibrosis of the hyaline type. Spirochetæ pallida were not demonstrable. The degenerative changes as studied in other portions of the aorta not involved in aneurysmal formation showed that they occurred

Fig. 1. Multiple aneurysms of ascending aorta.

about the openings of the vasa vasorum. The question of interest is the etiology of the condition. Is it to be considered specific in the sense that it is due to the spirochetæ, or is this particular case one of non-luetic origin?

The second specimen, a lymphosarcoma of the gall-bladder, gave the usual history of obstructive jaundice. The patient, a female of 55 years, entered the hospital suffering from a grad-

ually increasing jaundice of five weeks' duration. Physical examination showed an obese individual with fluid in the left chest as demonstrated by puncture, and a mass palpable in the general region of the gall-bladder and extending over into the region of the spleen. During the last few days of her life she gave evidence of partial intestinal obstruction, which was thought to be due either to a neoplasm of the large gut, or possibly to the pressure caused by the rapid return of the ascitic fluid. A second abdominal puncture was made, after which the patient gave the clinical appearance of a severe internal hemorrhage, and died.

At the autopsy it was found that the chest, aside from the presence of a few ounces of bloody fluid in the right pleural cavity, was negative. Upon opening the abdomen about two gallons of a very bloody fluid escaped and with it three recent blood-clots each as big as a hand. Careful search showed the hemorrhage to be from an arterial muscular branch which had been pierced by the aspirating needle, the subsequent bleeding having drained into the abdomen. The other intra-abdominal pathological lesions not associated with the new growth were multiple uterine fibroids, a chronic appendicitis with adhesions, and a mild degree of interstitial nephritis.

The chief organs of interest were the liver and gall bladder. The entire liver was enlarged and deeply jaundiced, and the falciform ligament was thickened and evidently invaded by some new growth. Upon turning back the liver the gall-bladder was found to be approximately three times its normal size (Fig. 2) and golden yellow in color. Upon tracing the gall-bladder along to the common duct and from there to the intestine it was seen that this enlargement was uniform and continuous, passing beneath the small intestine and connecting with a diffuse new growth which completely encapsulated a small spleen, and which, leaving the peritoneal surface of the left leaf of the diaphragm untouched, invaded the body of this muscle and projected into the left pleural cavity. The lumen of the gall-bladder was not appreciably narrowed, though the walls of the viscus were very thick. The jaundice was explained by a pure cholesterin stone

found in a small pocket at the beginning of the common duct. No gross metastases were demonstrable in any organ of the body. Where the gall-bladder was in direct contact with the liver, extension of the growth had occurred. The intestinal canal was normal except at the splenic flexure where the mass previously described caused a marked narrowing of the lumen by pressure from without.

FIG. 2. Lymphosarcoma of gall-bladder.

Microscopical examination showed the tumor to consist of a dense mass of round cells about the size of a lymphocyte, with a deeply staining central nucleus, and a narrow zone of protoplasm. These cells were arranged without any definite architecture. Invasion of the liver and diaphragm was demonstrable. The vascular supply of the tumor was poor and areas of necrosis were common.

The current opinion is that malignant tumors of the gall-bladder are relatively frequent. Why this opinion should be prevalent is not clear, particularly since Williams has shown that of 31,027 autopsies on cases dying of malignant tumor but 66, or 0.2 per cent., had the tumor primary in the gall-bladder. Similarly the etiological factor of gall-stone incidence is also grossly overestimated. It has been estimated that 10 per cent. of all

FIG. 3. Carcinoma of appendix. Gross appearance.

adults past the age of 30 years have gall-stones as shown by autopsy. If these figures are correct, then the incidence of gall-bladder malignancy must be very much higher before any etiological connection can be established between gall-stones and cancer. In the experience of the Lenox Hill Hospital and Lincoln Hospital during the past ten and five years respectively there have been but three primary malignant tumors of the gall-bladder out of a total of 3,129 cases of neoplasm, a percentage of ap-

proximately 0.1 per cent. While the most frequently described tumor of the gall-bladder is a carcinoma, there are recorded in the literature cases of sarcoma of this viscus, notably fibrosarcoma, myxosarcoma, and osteoid sarcoma. We have not been able, however, to find the record of another lymphosarcoma.

The third specimen is an unusual type of carcinoma of the appendix. The clinical history in brief is that a male, aged 30 years, was admitted to the hospital with the evidences of an acute appendicular inflammation. At the time of operation the peculiar appearance of the appendix attracted the attention of the surgeon who suspected a tuberculous involvement. In the gross (Fig. 3)

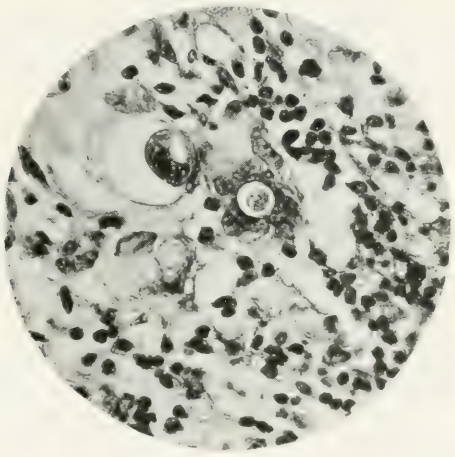

FIG. 4. Carcinoma of appendix. High power showing goblet cells in deep tissues.

the appendix appeared as a tube 7 cm. in length with a much congested peritoneal surface. On section the wall was found to be of varying thickness, its greatest diameter being 0.5 cm. Upon opening the lumen of the organ the mucosa appeared as a thick layer from which projected five small polyps, the largest measuring 0.25 cm. in length. The lumen of the lower third of the organ was obliterated.

Microscopical examination of the tip of the appendix showed the usual changes of an obliterative process. Sections taken

through the upper levels so as to include the polypoid-like projections showed a mucosa with papillary outgrowths covered with a high columnar epithelium which evidently secreted mucus. The deeper layers of this epithelial covering had penetrated the muscular coats of the organ and atypical gland-like structure could

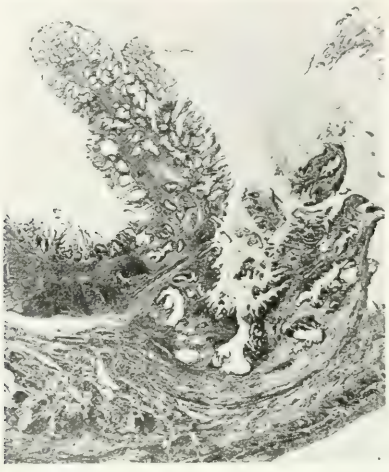

FIG. 5. Gelatinous carcinoma of appendix.

be demonstrated in the muscular coats, while single cells lying free in connective tissue spaces were common (Figs. 4 and 5). We have in this case apparently an early carcinoma of the mucoid type.

Discussion:

DR. MOSCHCOWITZ: I would like to make a few comments on the case of carcinoma of the appendix. I have seen a large number of carcinomata of the appendix, twenty, at a conservative estimate, but this is the first case of colloid carcinoma of the appendix that I have ever seen. As you know, the prognosis in carcinoma of the appendix is better than that for carcinoma involving any other organ of the body. As a matter of fact, the incidence of recurrence or metastasis of carcinoma of the appendix is rare, as far as I know the only cases in which it has been recorded are the adenocarcinoma of the colloid types. As Dr. Rohdenburg has said, the French authors have described some.

DR. LAMBERT: In the second case, was the final diagnosis lymphosarcoma or just an ordinary round-cell sarcoma? It is difficult to interpret the tumor from the morphology of the cells as seen in the lantern slide.

Dr. Rohdenburg: I took the tumor to be a lymphosarcoma of the gall-bladder; not a round-cell sarcoma; the cells are very uniform throughout the entire tumor and resemble a lymphocyte absolutely, except that they are just a trifle larger.

MYOSARCOMA OF UTERUS; ACTIVE TUMOR GROWTH BENEATH SKIN LESION CURED BY X-RAY

D. S. D. JESSUP, M.D.

This case is one which occurred on the service of Sloane Hospital. The operation was for a fibroid, and the specimen showed a small uterus removed without the cervix with a moderate sized fibroid situated beneath the mucosa. In the gross, it showed nothing unusual; but the microscopical appearance made it noteworthy as a pathological specimen. The mucosa over the surface of the submucous fibroid is very much thinned out, and beneath this, along the periphery, are the cells one would expect to find in a myoma. Within there are very large cells, increasing in size up to cells that are from two to ten times the size of ordinary muscle cells. This type of tumor has been described under a variety of names and has led to considerable discussion as to whether it was a pure sarcoma or a form of degeneration, or whether it was a malignant form of myoma. This particular specimen stained with Mallory's special connective tissue stain showed very large tumor cells, multinuclear in many instances, and having the characteristic structure of muscle cells, taking the grey stain rather than the pink in the surrounding connective tissue, and apparently of muscle origin, so that whatever it may be called, it seems to be a tumor of smooth muscle origin. This type of growth is recognized only microscopicaly, as it is not suspected either in the gross or clinically. It has been estimated that a considerable number of fibroids, if sectioned, would show these changes. Clinically, these tumors do not appear to be very malignant, as compared with what we expect of malignant sar-

comata, but a growth of this type, with many mitoses in the enlarged muscle cells and with giant cells, certainly has the morphological appearance of malignancy. In connection with this tumor, and as illustrating the capabilities of muscle cells to develop malignant tumors, Dr. Wood has loaned me a collection of slides illustrating a tumor of the prostate developing from the striated cells.

The second case is one of interest from the clinical side as well as from the side of therapeutics. It is a specimen of skin lesion entirely healed by the use of the *x*-ray; but with an actively growing carcinoma beneath the skin. The patient's history was finally obtained before the second operation, which was for the removal of the primary growth. The fact that she had another tumor was then brought to the doctor's attention by a lump which had appeared in her groin. It was learned that she had been treated in one of the downtown hospitals for a swelling of her foot, which had not improved, so she had come to this other clinic for treatment of the unhealed wound, and a diagnosis was made of perforating ulcer. She disappeared and at that time had a lump in her groin. Treatment did not help her, and she was turned over to the *x*-ray department for a cure of the ulcer on her foot. The *x*-ray produced the appearance of a nævus—soft, having a bluish tinge here and there as in a partially cured nævus. The *x*-ray treatment was not done with the idea of curing a malignant tumor, but of treating an unhealed ulcer.

Discussion:

Dr. Moschcowitz: I want to say the following to corroborate what Dr. Jessup said in regard to the frequency of myosarcoma of the uterus: if all the fibromyoma removed were sectioned more carefully I am sure that we would find that myosarcoma is more frequent than we now believe.

ECHINOCOCCUS CYSTS IN A MONKEY

ROBERT A. LAMBERT, M.D.

(From the Department of Pathology, College of Physicians and Surgeons, Columbia University)

The specimen consists of a *Macacus rhesus* monkey, which died in the Bronx Zoölogical Garden. It was sent to Professor Huntington for anatomical study, but, finding a striking pathological condition present, he kindly turned the animal over to me. The abdomen was enormously enlarged, and full of cysts. These were attached to the peritoneum everywhere, but especially over the omentum, mesentery, and lower surface of the liver. Only the capsule of the liver was involved, there being no cysts anywhere in the substance of the organ. There were, also, no cysts in the spleen, kidneys, or thoracic organs. The cysts grossly resembled in every way those which are so commonly found in pigs, and were not different from the multilocular cysts of the liver occasionally encountered in man. The larger cysts contained daughter-cysts, and inside were the so-called granddaughter cysts. Microscopic examination of the fluid in the cysts revealed numerous scolices, which in the fresh state were seen to be definitely motile. Adherent to the inner walls of the sacs, the scolices could be seen with the naked eye as tiny, yellow specks. After autopsy the specimen was placed in a very cold ice-box overnight, and on the following morning, some of the cysts were fed to two dogs. These were kept for six weeks and then killed. In the feces of these dogs, during life and after death, we failed to find any ova of *Tænia echinococcus,* and a careful examination of the gut, at autopsy, revealed no worms. It is possible that the animals were not kept long enough, since some investigators have found that the worms do not develop before eight or nine weeks. It is also possible that these particular dogs were not favorable hosts, although it has been noted that the percentage of dogs which can not be infected is small. The fluid in the cysts was partially frozen in the cold ice-box, so that the

chilling of the worms may have been a factor in preventing infection.

It may be stated that echinococcus infection in monkeys is not rare, especially among those which have been fed on fresh meat in zoölogical gardens.

PARAGENITAL TERATOMA

L. W. STRONG, M.D.

This tumor should be described as a paragenital teratoma, since its exact origin is indeterminate. It was located at the fimbriated extremity of the right tube, its pedicle being the fundibulopelvic ligament in which ran a branch of the ovarian artery. It was adherent below to the sigmoid, and extended up almost to the umbilicus. The tumor was 24 cm. in diameter, and the outer surface was smooth and showed many cysts of varying size, with membranous walls. On section the tumor was of a honeycomb appearance, but was in the main solid. No sebum, bone, hairs, or teeth appeared in the gross specimen.

The sections showed a multiplicity of structures, the stroma being largely fibroblastic, with many large areas of myxomatous cells which were stellate. In this stroma appeared elements of all three germinal layers, mostly of an embryonic type. There was squamous epithelium of all degrees of maturity, and this changed abruptly into other types, such as stratified, ciliated, papillated, beaker cells, and particularly abundant neuroepithelium. In this latter were areas of dense pigment succeeded by papillations, evidently representing choroidal villi. In one location there was a structure strongly suggesting the eye vesicle. Nonmedullated nerve fibers were abundant and large ganglion cells lay between them. Hair follicles, embryonal fat cells, and cartilage of all stages of ripeness occurred. There was one circular structure suggestive of a definite organ formation, with a lumen lined by high columnar epithelium and surrounded by a

circular muscular layer. I should be glad if someone would identify this. No ovarian tissue was found. In the region of the choroidal villi were spaces covered with delicate membranes suggestive of ventricular cavities. Naturally a teratoma in this location would suggest an ovarian origin, but in this case both ovaries were intact and not physically connected with the tumor. It is possible that the tumor arose in the right ovary and became snared off, or that it was a teratoma of an accessory ovary or paroophoron, or finally that the misplaced germ-cell was embedded in the broad ligament. Pfannenstiel in Veit's Handbuch mentions the possibility of snaring off, since teratomata are usually pedunculated. The case of Fraenkels, which Pfannenstiel mentions, turns out to have been a dermoid. A large and rapidly growing teratoma would most likely become necrotic if thus snared off. There is no possibility of demonstrating whether there was an accessory ovary or not, but tumors of accessory ovaries have been reported. As to the primary location in the broad ligament, although dermoid cysts have been reported in the tube (Orthmann) no teratomata have, as far as I can find.

Discussion:

Dr. Larkin: The consensus of opinion apparently is that paragenital teratomata represent superfetations. I was extremely interested in the picture which the doctor asked someone to interpret. I saw a similar picture in a teratoma of the ovary, and I think it represents a very early development of the bronchi. The character of the epithelium lining it, and the condition of cellular infiltration in the submucosa, with the development of muscular tissue, would represent perhaps some part of the bronchial tree. This is rather unusual in teratoma, as the tissues we usually see are the low embryonal developmental tissues, but Dr. Strong has just happened on a specimen which I think is remarkable for the development of the various structures.

Dr. Moschcowitz: I am free to confess that I have never seen a teratoma in which there is such a wonderful variety of tissues as in the one presented, and I have never seen one in which there is such an advanced organization of an organ. To me the theory of parthenogenesis seems most applicable to explain this remarkable teratoma, which is only another way of saying what Dr. Larkin has just said.

CONGENITAL ATRESIAS AND STENOSES OF THE GASTROINTESTINAL TRACT

A. V. ST. GEORGE, M.D.

(From the Pathological Laboratory, Bellevue and Allied Hospitals, Dr. Douglas Symmers, Director)

Congenital malformations of the gastrointestinal tract are not infrequent post-mortem findings, although certain types, especially those occurring in the small intestine and esophagus, are not very often encountered. Of the latter it has been suggested that more cases actually do occur but because of the insufficient number of autopsies, many are overlooked. Anal atresia being an external malformation, is easily recognized, and hence this condition is mentioned as the one most frequently met with. Gant states that some form of malformation of the rectum or anus is found at least once in every ten thousand births.

Congenital atresias or stenoses may occur anywhere in the gastrointestinal tract, but their usual site is the esophagus, the duodenum, the rectum, or the anus. Cases with multiple points of atresia have been described, and combinations with involvement of the esophagus and duodenum, or of the duodenum and anus are also found. (Hypertrophic pyloric stenosis, although probably due to some faulty development, does not manifest itself until a considerable time after birth and is therefore not included in a discussion of the congenital malformations.)

The etiology of these malformations is still a matter of dispute; but as we obtain a clearer knowledge of the embryological development of the human body, it becomes increasingly apparent that many and probably all atresias of the alimentary canal are due to failures in development of the primitive gut; a view that is held by such men as Keibel, Schafer, Kreuter, and Bailey and Miller. Congenital syphilis, fetal peritonitis, thromboses, volvulus, inspissated meconium, intussusception, ulcers, etc., may play a rôle now and then in an isolated case, but from the small number of cases of atresia in comparison with the large number

of cases of congenital syphilis, peritonitis, etc., found in the newborn, we must conclude that these factors are of but small importance.

The following specimens were obtained from patients in Bellevue Hospital. The case of congenital atresia of the duodenum has been made the subject of an extensive report and published elsewhere and only a brief account is given here. (Congenital Intestinal Obstruction, with the Report of a Case—*Am. Jour. Dis. Child.*, 1918, xv, pp. 354–361.)

The white female child was born of healthy parents; family history negative. At birth there was some oozing of blood from the nose and mouth. The child was given the breast, but shortly after vomited some dark stained fluid. There was an attempt made to control the hemorrhage by injection of horse serum, and later on, of the blood of the mother, but the child continued to bleed with only slight periods of intermission. Repeated nursing only resulted in vomiting; during one of these attacks, on the sixth day after birth, the child died. Enemas and the administration of cathartics brought no relief. At autopsy the following was found:

Anatomical diagnosis: Congenital atresia of the duodenum; hemorrhagic disease of the newborn; icterus neonatorum (obstructive jaundice); uric acid infarction of the kidney; pial edema, marked.

The greater portion of the large and small intestines was collapsed. There were numerous hemorrhages in the parietal and visceral peritoneum of the intestines, stomach and liver. The stomach and greater portion of the duodenum were markedly distended with semi-fluid food residue and gas. Approximately 9 cm. from the pylorus there was a complete closure of the duodenum so that nothing could be pressed through it. Beyond this closure the intestine again assumed a fairly normal shape for a distance of 20 cm. when again an apparent complete closure was encountered (Fig. 1). The remainder of both the large and small intestine was completely collapsed. The organs were in their normal position; there was no rotation or transposition of

any portion of the gut. Microscopic examination of the closure at the duodenum revealed an absence of the intestinal lumen and replacement by a fibrous band. That in the jejunum showed a narrowed lumen filled with blood clot.

The second case, a white female child, was born of healthy

Fig. 1.

parents; family history negative; normal delivery. A few hours after birth the child was given some water which it regurgitated a short time after. When the child was put to the breast, it would nurse eagerly but immediately thereafter vomited the food. Gavage was attempted but the tube could not be passed down the esophagus. The passage of an esophageal bougie, also,

was impossible. The child died nine days after birth, having become considerably emaciated. At autopsy the following was found:

Anatomical diagnosis: Esophageal atresia (congenital); tracheoesophageal anastomosis (congenital); uric acid infarction.

The tongue was smooth; the tonsils were small. At a distance of 4 cm. from the pharynx there was a complete closure of the esophagus so that not even water passed through it. The larynx was normal. On inserting a probe through the larynx and down into the trachea, it easily slid through a tube composed of muscle tissue which terminated in the cardiac orifice of the stomach. Passing water from the cardia of the stomach upward, it was found to flow out of the larynx so that there was a definite and well established tracheoesophageal anastomosis. The remainder of the gastrointestinal tract was normal and contained a small amount of meconium. The bronchi were normal (Fig. 2).

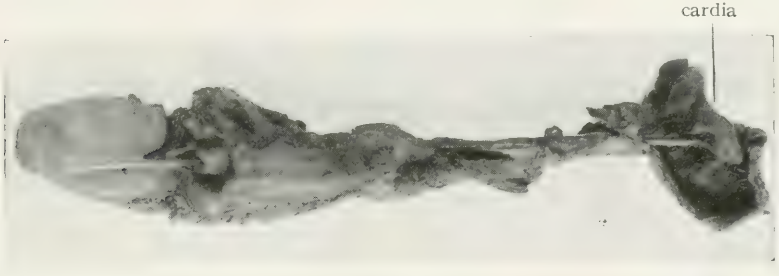

FIG. 2.

In connection with the second case, it is worth while to mention Whipham and Fagge's article, in which they classify the malformations of the esophagus into the following five groups:

1. Congenital absence of the whole esophagus (a very rare condition).

2. Bifurcation of the esophagus with junction of the two parts towards the lower end (only one recorded case known).

3. Congenital atresia in which the esophagus is divided into two parts (the least rare of all the varieties).

The upper end terminates blindly in a more or less expanded

cul-de-sac. The lower opens above into the trachea or into one of the bronchi and below in a normal manner into the stomach. The two segments of gullet may be connected by a fibrous or muscular band.

4. Stricture caused by a fold of mucous membrane projecting into the lumen of the tube like a diaphragm. This may occur at the upper portion of the esophagus, immediately below the pharynx, or near the lower end.

5. Congenital stenosis of the lower end of the esophagus.

The cause of atresia of the duodenum is described by Kreuter as being due to a temporary closure of the lumen of the primitive gut, occurring early during fetal life and brought about by epithelial hyperplasia. Failure to reopen produces the malformation. Atresia of the esophagus is stated by Bailey and Miller to represent an important separation between the anlage of the respiratory system and the primitive gut.

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Discussion:

Dr. Moschcowitz: I have been rather interested in the problem of congenital stenosis of viscera, having seen quite a number of such cases. Many years ago I dissected a specimen of congenital atresia of the esophagus in which the lower end of the esophagus entered the trachea. It seems curious that this form of congenital atresia of the esophagus, the most complicated of all, should be the most common. At that time I tried to obtain an embryological explanation of this type of atresia, and even with the aid of Dr. George S. Huntington, I was unable to find a satisfactory explanation. A circumstance worth mentioning is that congenital stenoses of the viscera commonly occur at those situations where an embryological bud has been given off from the lumen; in the aorta, for instance, opposite the entrance of the ductus arteriosus; in the duodenum, opposite the common bile duct; and in the small intestine, opposite the exit of Meckel's diverticulum.

PHLEGMONOUS GASTRITIS: REPORT OF A CASE

F. I. SHATARA, M.D.

Phlegmonous gastritis or submucous gastritis is a very rare condition first described by Benel in 1656. In 1904, Robson and Moynihan collected eighty-five cases, and up to the present about one hundred cases have been reported.

The condition is an acute suppurative inflammation affecting the submucous coat of the stomach and sometimes extending to the mucous, muscular, and serous coats. It is sometimes primary, but usually secondary, occurring during the course of such diseases as typhoid fever, scarlet fever, smallpox, and puerperal septicemia. Two forms have been described, the diffuse and the circumscribed.

The etiology is not definitely known; several theories have been advanced, of which the bacterial seems to be the most plausible. Streptococci have been the commonest microörganisms present; colon bacilli have been occasionally demonstrated. In the case here reported, streptococci were demonstrated by the Gram-Weigert stain. In secondary cases it is probable that the infection is carried by the blood stream. It is held by some that the invading organisms find their atrium through lesions in the superficial epithelium, and ulcer, cancer, and traumatism are consequently given as predisposing causes. Symmers at Bellevue Hospital failed to produce the lesion in animals by feeding them ground glass and inoculating streptococci and pneumococci.

The pyloric region of the stomach seems to be the site of predilection, although the process involves the entire stomach, and sometimes the esophagus, diaphragm, pleura, pericardium, peritoneum, spleen, kidneys, liver, and gall-bladder are involved.

The onset is sudden with symptoms of intense gastritis, profound general disturbance, and a rapidly fatal termination. Sometimes the symptoms of general peritonitis, as in the case here reported, are superimposed, and it is probable that many cases are thus wrongly diagnosed and overlooked. Pain, epi-

gastric tenderness, nausea, vomiting, chills, fever, and hiccough are the outstanding symptoms. Hiccough was marked in this case. Collapse and coma are frequent terminal events. The course is usually from one to seven days.

The diagnosis during life is at best a guess. Chvostek is said to have succeeded in making one such diagnosis. The treatment is symptomatic, except in the circumscribed form with a localized abscess, where surgery is indicated.

The following is a brief report of a case admitted to the Englewood Hospital:

C. W., colored, aged 29, laborer, American, admitted January 19, 1918; died January 21, 1918.

Family and Personal History: Negative.

Present Illness: Chief complaint: pain in abdomen, headache, vomiting, and anorexia; duration one day; onset sudden.

One month previous to admission, the patient applied for treatment at the dispensary. He then complained of a vague abdominal pain, general in character. The pain was intermittent in type, increasing usually after meals, but never severe enough to confine the patient to bed, nor to interfere with his appetite. His bowels were constipated. There was no history of vomiting, gaseous or sour eructations, or jaundice. Physical examination at that time revealed a slight abdominal tenderness, more marked on the right side. There were no palpable masses, nor any definite rigidity. Over the aortic area there was a faint systolic murmur transmitted to the carotids, but no other signs of cardiac trouble. The patient was at that time advised to enter the ward for observation, but this he neglected to do. One week later he came back to the dispensary complaining of the same vague abdominal pain. A Wassermann was suggested and the patient was again advised to enter the ward, but he again refused.

On the morning of January 19 the patient felt worse. He was unable to take his breakfast, and this he interpreted as a sign of severe illness. He applied to the dispensary at 9 a.m., complaining of headache. He was given half a grain of codeine and ordered to bed. At 10 a.m. the patient vomited. At 4 p.m. his condition grew worse, his abdominal pain increased, and he felt nauseated. The abdominal tenderness was marked, and there was definite rigidity, more marked on the right side. There were no signs of fluid in the abdominal cavity. A ruptured gastric ulcer was suspected and the patient was admitted to the ward. He then had a white blood count of 15,000, temperature of 101.6, pulse 100, and respiration 22.

He slept about seven hours that night. Next morning he repeatedly vomited green fluid. At 8 a.m. he had a white blood count of 27,400, polymorphonuclears, 92 per cent., large mononuclears, 8 per cent.; temperature 103.2; pulse 108, and respiration 24.

He was operated on at 11.30 a.m. About 2 ounces of serosanguinous fluid were found in the peritoneal cavity. No definite perforation was located, and a posterior gastroenterostomy was unnecessary and impossible on account of the excessive thickening of the stomach wall. A gastrostomy was performed and it was noted that the stomach wall had lost its elasticity and become so indurated that it was difficult to suture to the gastrostomy tube. Two cigarette drains were inserted into the peritoneal cavity to provide for drainage, and the patient was sent back to bed. His general condition then was fair, pulse 116, and respiration 24. Murphy drip and pituitrin were given. About 11 p.m. the patient became very restless and thirsty and complained of pain in the operative area. He also had a hiccough, and there was a bloody discharge on his dressing. An irrigation with warm saline through the gastrostomy tube was performed, the return was at first bloody, but later became clear. The dressing was changed. The patient's temperature had gone up to 104.41, pulse 128, and respiration 26. He slept about four hours. Next morning nutritive enemas were started. In the drainage bottle to which his gastrostomy tube was connected there were 16 ounces of wine-colored fluid. About 8 a.m. the patient's temperature went down to 102, his pulse fell to 118, but his respiration was accelerated to 32. About 3 p.m. his abdomen was distended and tympanitic, but there was no evidence of free fluid. He was given an alum enema with good result. About 4 p.m. he complained of headache and was given an ice cap. He took a piece of ice out of the bag and swallowed it. His general condition grew worse, his pulse rose to 138 and was thready; he was given stimulation, but did not respond, and died at 7.50 p.m.

Summary of Autopsy Findings (autopsy limited to abdomen): There was about a quart of bloody fluid in the general peritoneal cavity. The intestines were distended and from numerous pinpoint perforations throughout the gastrointestinal tract brownish, fluid gut contents could be squeezed out. The intestinal wall was congested but not markedly thickened. No miliary tubercles were visible on the serosa. On opening the gut, numerous pinpoint perforations were discovered. These were barely visible and gave the impression of perforations, produced by swallowing ground glass. These perforations were surrounded by areas of congestion, but no ulceration or surrounding induration was demonstrable. They were scattered throughout both the small and large intestine. The mesenteric nodes were enlarged. The stomach showed a different picture. The wall was markedly thickened, measuring from one third to one half inch. The mucosa was apparently obliterated and the stomach looked and felt leathery. There were no ulcers, but about half a dozen pinpoint perforations similar to those in the intestine were present. No surrounding areas of congestion could be differentiated, due to the general uniform thickening of the stomach wall.

The liver was of a pale opaque color; its substance was soft and mushy, reminding one of the liver in cases of acute yellow atrophy. The gall-bladder showed signs of cholecystitis.

The spleen showed a perisplenitis (Zucker Kuss). No tubercles were visible. The splenic tissue appeared normal, except for a slight congestion.

The remaining organs were negative.

Microscopical examination of sections taken from different portions of the stomach revealed a diffuse infiltration of the stomach wall with leucocytes. This infiltration was most marked in the submucosa, which was thickened and edematous. The blood vessels were engorged, and there was a marked perivascular infiltration. The other coats of the stomach wall showed a similar though less marked infiltration. The process was diffuse, not being localized to any one portion of the stomach wall.

Gram-Weigert stain demonstrated the presence of streptococci, chiefly in the submucosa.

Discussion:

Dr. Moschcowitz: May I ask what the two perforations in the intestine are due to? Did you demonstrate ground glass?

Dr. Shatara: No, sir. I do not know, except that they may be due to little abscesses which have perforated through the serosa.

Dr. Larkin: Anyone having much experience in autopsies knows that suppurative gastritis is a very rare condition. In my own personal experience I have seen but three cases. At Bellevue a number of years ago we had a case in which we found the condition accidentally. This corresponds to the general experience. I also recall another case at the Presbyterian Hospital.

The condition of petechiæ accompanying suppurative gastritis generally depends upon a thrombotic condition of the blood vessels in the submucosa. The blood vessels are seen to be filled with minute bodies which look like organisms. One point which must be borne in mind is that in suppurative gastritis the mucosa is always preserved. This particular specimen shows that very well indeed, and I have in my possession two specimens in any part of which you will find the mucosa fairly well preserved. In this specimen the lesion is in the submucosa and it shows more edema than actual suppuration. Only in the musculature and serosa are thrombi found, which explains the clinical acuteness of symptoms leading to a diagnosis of acute peritoneal inflammatory conditions. I want to say a word about the bacteriology of suppurative gastritis. Up to the present time I do not believe any reliable work has been done as regards the causative organism. All kinds of bacteria have been found.

Dr. Moschcowitz: It seems remarkable that we should not find this lesion more often considering the frequency of ulcers of the stomach.

Dr. Shatara: In looking up the bacteriological side of the question, and the mode of entrance of the bacteria, it was brought out that the bacteria do not enter through the food, else we should find the condition oftener in association with cases of gastric ulcer and carcinoma. It seems to be the consensus of opinion that the bacteria are carried to the stomach through the blood.

A SIMPLE AND INEXPENSIVE METHOD OF PREPARING COLORED LANTERN SLIDES FOR TEACHING

ROBERT A. LAMBERT, M.D., AND ALFRED FEINBERG

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In teaching the course in pathology at the College of Physicians and Surgeons during the past few years, a projection apparatus, or epidiascope, has been used for demonstrating microscopic sections, photographs, drawings, etc. Recently, owing to structural alterations in the building, it was found impossible to darken the classroom sufficiently for satisfactory projection. We therefore had to resort to the use of lantern slides which, as you know, may be shown in a semi-darkened room. It occurred to us, however, that students, accustomed to stained histological preparations and colored illustrations, would not find the black and white lantern slide a satisfactory substitute for the projected stained section or colored picture. We therefore decided to use colored lantern slides, provided a simple and inexpensive method of preparing them could be devised. There are two types of colored lantern slides in common use, Lumière plates, and hand-colored slides. The preparation of Lumière plates was not attempted on account of the expense, and the recognized difficulties and unsatisfactory results of the method. The cost of hand-colored slides also appeared prohibitive, since the people who do this work as a business make a charge of a dollar to a dollar and a half per slide. We needed four hundred or five hundred slides, for which the cost, therefore, would have been around five hundred dollars. After some little experimentation, we found that lantern slides could be colored easily in the laboratory at small expense. The two methods, which have proved simple and practicable, can be described in few words.

The first method is practically the same as that ordinarily employed, except for a few short cuts and modifications. The

slide is printed light, but with as sharp definition as possible. Over this light positive, the desired colors are laid on with a fine brush. Japanese water colors are used. In illustrating microscopic sections, where generally a hematoxylin-eosin effect is aimed at, it has been found unnecessary to color the nuclei blue, —a tedious matter in low power illustrations,—since the difference between blue and the original black is not very noticeable. Cytoplasm, connective tissue, and muscle are colored pink. In many instances, a tone of pink applied rapidly over the entire slide produces the desired effect. To make a colored lantern slide from a colored drawing sometimes offers difficulty, since certain colors, particularly reds and yellows, come out very dark in the print, making it difficult or impossible to lay on subsequently the proper color. This difficulty has been overcome by touching up the negative so as to get a light positive, that may be easily colored. There are other small details which any intelligent laboratory assistant with good color sense will easily work out for himself. Illustrations of gross specimens are more easily colored than those of microscopic preparations, since there is generally less detail, and oftentimes a single tone over the entire picture will give a very pleasing effect. The shading in the original black and white gives the proper variations in depth of color. In this way, an ordinary uncolored slide, which may mean little to the students, can be converted into an impressive colored illustration in two or three minutes with little or no expense.

The second method consists in drawing colored pictures directly on the unexposed lantern slide plate. The plate is dipped into the fixing bath without previous exposure or development and then allowed to dry. On the film side of the glass, water color drawings can be made almost as easily and as rapidly as on paper. Such drawings project with great clearness and brilliancy, owing to the fact that practically no light is absorbed. Furthermore, the expense of photographing and printing is avoided. It may be explained that drawings can not be made on plain glass, and that something in the nature of a gelatin film over the glass is necessary. Such a film may be put on in the

laboratory, but we have found it advisable to use the ready prepared plates as indicated.

The first method has been used where we had on hand the illustrations which we wanted reproduced; the second has been used for illustrating microscopic preparations of which drawings and photographs have not been made, or for copying illustrations which can not be easily photographed.

Discussion:

Dr. Wood: I think Dr. Lambert is right when he says that color adds a great deal to teaching medical students by means of lantern slides, either of sections or gross material. We are never accustomed to black and white for microscopic sections, and our diagnosis depends very largely on our familiarity with stains. The method of coloring slides is a very old one, as Dr. Lambert says. To attempt projection of Lumière plates is a waste of time, for if you do have a good plate, it is too dark for use. Only one-tenth of the incident light passes through the slide. The Paget plates pass about fifty per cent., but they are technically difficult to make. There is a new process brought out by the Eastman Company which is not yet perfected. It promises well, but it is still difficult and uncertain. As far as I am concerned, I think the simplest way is to have the ordinary lantern slide hand colored.

Dr. Larkin: I think we all like to tell of our experiences in color photography. I thoroughly agree with Dr. Wood and all the other photographers who spend time on this worthy cause that our processes are far from perfect. I have something like one thousand Lumière plates covering many subjects, and though they are beautiful to look at through the bright sunlight, they are very disappointing when thrown on the screen. No matter how powerful the light is, poor results follow. This renders the Lumière plate unsuitable for teaching purposes. I have used a plate made by Fitzsimons of 75 Fifth Avenue. Fitzsimons maintains that only his developer should be used, and my own photographer, using Fitzsimons' developer, obtains more definition than when using the one advised by the Lumière people. With the plate which Fitzsimons has put out, we get very high color differentiation. One difficulty, however, is that when you put it into the lantern there is more or less of a haze over the picture.

Dr. Ewing: This is a very important subject because it deals with the methods of teaching medicine. At the next meeting of the American Medical College Association the main topic will be the discussion of pedagogical methods in medicine, and I feel that schools all over the country ought to investigate these methods, and standardize them as much as possible. It seems to me in this particular field there is an opportunity for great improvement in the material available in many schools in this country. There are sixty-two medical schools in the Medical College Association, and I am quite certain that not all of them have good material to demonstrate gross

and microscopical lesions. Now if it were possible for one firm to make one or two or three thousand lantern slides of gross and microscopical preparations it would be a very important addition to the material for teaching. Among other advantages, it might obviate certain idiosyncrasies which are apt to crop out from time to time in our teaching. Whether there is a commercial process which will furnish color plates for a small sum of money is questionable, but I think they could be cheaply produced by a technician employed by the College Association. Personally, I do not use colored lantern slides in teaching. I think my work falls down perhaps on that account. If you have over one hundred students you cannot give them all the desirable personal attention, and as we have always had small classes, I have limited myself to gross and microscopical material, a method which I think is distinctly better than photographs. When photomicrography first came out I was very conservative about its use, but the more you cater to the artistic color sense of an audience, the more they are interested. Yet there is no doubt that medical audiences are now pretty keen in interpreting ordinary photomicrographs, and I think they rather look forward to seeing them at a lecture, but for the teaching of medical students there is an advantage in colored slides.

Dr. Lambert: It has been very interesting and instructive to hear of the experiences of Dr. Wood, Dr. Larkin, and Dr. Ewing. I have learned quite a lot from them, especially what not to attempt in the way of color photography. In regard to what Dr. Ewing said about the relative value of microscopic sections and lantern slides in teaching, I should like to explain that in our teaching we use both lantern slides and sections. We find it difficult to demonstrate to each student all the things that he should see under the microscope, so that before or after they have studied the sections, a lantern slide demonstration is given by means of which the more important features can be pointed out to the class as a whole.

EXAMINATION OF THE BONE-MARROW AT AUTOPSY WITH A METHOD OF OBTAINING SPECIMENS

ROBERT A. LAMBERT, M.D.

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It may be safely stated, I think, that routine examination of the bone-marrow at autopsy is done in very few institutions, and that one rarely sees sections of bone-marrow except from cases where a definite lesion was suspected during life; such, for example, as carcinoma with metastases to bones, pernicious anemia,

leukemia, etc. This is true even in laboratories where practically every other organ or tissue of the body is carefully investigated grossly and microscopically as a matter of routine. The general neglect of a tissue of such obvious importance can hardly be attributed to a failure to recognize its importance. It might possibly be suggested, that on account of the difficulty in interpreting pathological changes in the bone-marrow, its examination is hardly worth while; but the same might be said of the adrenal, hypophysis, or even the spleen (and, in my opinion, with greater justification) organs, which in many pathological laboratories are regularly sectioned and studied. After examining the bone-marrow from several hundred autopsies, I have no hesitancy in saying that definite and easily recognizable pathological changes occur more often in this tissue than in many other organs or tissues, to which far more attention is generally given. I hope to present a review of this material before the Society at another time.

I believe that the real explanation of the general disregard of the bone-marrow lies in the fact that its investigation requires some extra trouble, and that the result of the effort to obtain specimens is not always fruitful or satisfactory. Specimens containing bone require decalcification, which often spoils the material for subsequent microscopic study. In view of the difficulty in obtaining satisfactory pieces of bone-marrow with the methods ordinarily employed, I have thought it worth while to describe a method which an experience of two years has proved satisfactory in every way. I should explain that it was at Professor MacCallum's suggestion that an investigation of the bone-marrow was made a part of the autopsy routine at the Presbyterian Hospital, and that the method described here is a modification of one that he proposed.

A lantern slide illustration will show the autopsy incision which permits an examination of the marrow of the humerus. The incision has the added advantage of giving a very free exposure of the neck organs without entailing a cut in the skin of the neck, to which objection is often made, especially in

autopsies on women. The first part of the incision starts on the right shoulder or upper third of the right arm, passes upward, forward, and then downward, making a broad curve, the lower border of which crosses the sternum about the third rib; the cut ends on the opposite shoulder. A second median, vertical incision begins at the point where the first crosses the sternum and ends at the pelvis. Dissection of the flaps on one side exposes the upper third of the humerus. Two parallel saw cuts are then

Fig. 1.

made; the first just below the surgical neck, the second only two inches below the first. In each, the saw is carried at least halfway through the bone. Then with hammer and chisel, the bone flap between the two saw cuts is lifted off, exposing the marrow cavity. Care should be exercised not to chisel deeper than the saw line, otherwise the bone will fracture. The marrow is viewed in situ; the color and consistence are noted; and then blocks of it are removed with a bone curette, which should be of a size just admitting insertion into the cavity. Though a very friable

tissue, bone-marrow can be taken out in small solid chunks. These are dropped immediately into small bottles containing fixing solution (Zenker's fluid and formaldehyde), and kept separate from the other tissues throughout the entire process of embedding. We find very convenient the ordinary fifteen c.c. bottles with wide mouth. After being well corked, these are dropped into the big jar containing the other organs from the autopsy, and the necessity for separate labeling is thus avoided. By using a small curette, pieces of bone-marrow can be secured from the head of the humerus, as well as from the shaft several cm. below the opening made. In the specimens obtained in this way, there are generally only a few spicules of bone, and decalcification is not necessary, especially if the tissue is fixed in Zenker's fluid, which tends to soften any small bits of bone that may be present.

Discussion:

DR. EWING: I think it is essential to examine the bone-marrow as a routine procedure. I always have done so and take as a rule a rib and vertebral body. It seems to me that if you ran that cut up to the head of the bone you would get a better idea of the changes in the bone-marrow which may be limited to the ends of the bones. Of course, the main difficulty with the study of bone-marrow is that you cannot examine all the bones in the body.

DR. LAMBERT: We usually do make the opening in the bone a little higher up than is indicated in the picture, so that it is possible with a curette to obtain specimens of marrow from the diaphysis of the bone. Dr. Ewing is probably right in pointing out that bone-marrow from more than one part of the skeleton should be examined, and I believe it would be well to take pieces from one of the flat bones, such as the vertebræ, as well as from the humerus or femur, as has been our custom. However, on the basis of the studies of the marrow in more than 150 cases, I am inclined to believe that bone-marrow changes are generally diffuse, and that an examination of even a single specimen from one long bone gives a fairly accurate idea as to what has been going on in the entire blood-forming apparatus.

DR. EWING: Is there any objection to this cut on the part of the undertaker?

DR. LAMBERT: We have never received any protests, either from the undertakers or from the relatives of the deceased. On the contrary, several undertakers have expressed their approval. I might explain that we are careful to leave the carotids intact, even when there is a thorough examination of the neck organs, and that to each carotid a string is attached, the free end of which passes out through the autopsy incision. This makes it an easy matter for the undertakers to find the vessels, and they seem particularly grateful to us for our attention to this point.

ADENOCARCINOMA OF THE KIDNEY IN A CHILD; A FIBROMA AND A FIBROMYOMA OF THE INTESTINE; AN UNUSUALLY LARGE CARCINOMA OF THE STOMACH

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Malignant tumors of children, particularly in the first few years of life, are far from uncommon, and the only excuse for showing this specimen is that, contrary to the usual type found, not alone in children, but also in adults, this tumor is a carcinoma. The clinical history is, briefly, that a male child, ten days after a normal and uneventful birth, was noticed to have a small mass in the right kidney region. This mass grew rather rapidly and at the age of two months reached its present size. At this time the child was operated upon and though the tumor was successfully removed death occurred two days after operation. The tumor measured when fresh $13 \times 10 \times 8$ cm. and was definitely encapsulated. Microscopical examination showed a typical papillary adenocarcinoma.

Benign tumors of the intestine are still sufficiently rare to warrant recording them as they occur, fibromata being the most infrequent, and lipomata the most common.

The first of these cases, a fibroma of the ileum, occurred in a male, aged 50, who presented the clinical symptoms of an acute intestinal obstruction. Upon operation, approximately a meter and a half of gut was resected, the tumor being so situated that the removal of the segment of gut containing it resulted in the destruction of the blood supply for this large section. The gross specimen is that of a pedunculated growth, 7 cm. in its largest diameter. This growth had obstructed the lumen of the intestine and, becoming partially turned on its pedicle, had become inflamed. The patient subsequent to the operation contracted a pneumonia from which he died during the fourth week after

operation. Microscopical examination of the tumor shows a rather cellular fibroma, the superficial portions of which are partially degenerated and infiltrated with polymorphonuclear leucocytes. The growth has apparently arisen from the submucosa.

The second case also occurred in a male, aged 35, the clinical symptoms being an acute hemorrhage of great severity. The tumor was found in the first portion of the jejunum. The growth measures 6 cm. in its longest diameter, which incidentally is at right angles to the long axis of the intestine. Microscopically the tumor consists of bundles of smooth muscle cells and a few fibroblastic elements. Tumors of this type are rather common.

The specimen of gastric carcinoma is remarkable for its size, measuring, when fresh, 18 cm. in its greatest diameter. It is approximately round, non-ulcerated, and projects above the mucosa for a distance of 3 cm. Histologically it apparently started as an adenoma, the base of which has become malignant. The patient from whom it was removed presented the usual clinical picture of a gastric carcinoma, and his post-operative recovery was uneventful.

Discussion:

Dr. Strong: Did you find any cases in the literature of carcinoma of the kidney of that type in children?

Dr. Rohdenburg: Yes, there are others. I was able to find five or six without much trouble, and if I had looked more carefully I believe I could have found more.

Dr. Strong: The common mixed tumors generally have a variety of appearances, and perhaps one part may overgrow another.

Dr. Rohdenburg: That is true, but sections taken from different parts of this tumor showed only a papillary adenocarcinoma.

Dr. Lambert: I should like to ask whether the tumor of the intestine which Dr. Rohdenburg described was a pure fibroma or a fibromyoma.

Dr. Rohdenburg: The piece I was allowed to remove was a fibroma.

Dr. Moschcowitz: Many years ago I showed a specimen here which was practically identical with the one presented to-night. It occurred in a woman who gave all the symptoms of intestinal obstruction. At operation an intussusception was found at the site of the tumor.

Dr. Lambert: I have seen a good many fibromyomata of the gastro-intestinal tract, most of them in the stomach wall, but I have never encountered a pure fibroma. These fibromyomata are generally very small growths, and are probably often overlooked.

Dr. Rohdenburg: Fibromyomata seem to be more common in the stomach. As regards benign tumors, I do not think that they are as unusual as people suppose, to judge from the surgical material for the past eight years. During this period we have had 364 intestinal tumors, and two and a a half per cent. of them have been benign.

Dr. Moschcowitz: The specimen of gastric carcinoma reminds me of a case I saw when a student. The patient felt well, except for slight weakness, and worked up to three or four days before admission. Death occurred soon after, and at autopsy the cancer involved practically all the tissues from the cardia to the pylorus; there were metastases in the glands, the liver, and even in the spleen.

Dr. Lambert: Evidently the point which Dr. Rohdenburg intended to emphasize was not that this specimen of carcinoma of the stomach was a particularly large one, but that it is unusual to see so large a tumor removed surgically. No doubt, everyone has seen much larger tumors than this at autopsy. It seems to me very doubtful that so large a tumor could have been removed completely enough to prevent recurrence.

TWO CASES OF PRIMARY CARCINOMA OF THE LUNG

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(From the Department of Pathology, Cornell University Medical College, New York City)

The salient points in the histories and autopsy findings of the two cases of primary carcinoma of the lung reported are as follows:

First Case: The patient was a young man, only 24 years old, who was admitted to the hospital on February 14, 1918. He had been troubled with shortness of breath for a year, which, during the past few weeks had been growing gradually worse. Lately he had coughed at intervals. Five weeks before admission the patient had noticed that his thyroid cartilage was considerably displaced to the left, and at about the same time he observed a swelling in the right supraclavicular region, which was nodular, hard, nonfluctuating, and not movable.

The expansion of the chest was diminished on the right side, especially in the upper part. Anteriorly there was flatness on percussion at the right apex, extending down to the second rib. Posteriorly the flatness extended from the apex to the middle of the scapula.

During his stay in the hospital his chief complaint was respiratory difficulty. Just before his death, which occurred on March 3, 1918 (two and one half weeks after his admission) he was extremely orthopneic.

At autopsy, performed the day after his death, there was seen to be a hard, flat swelling beneath the skin, about 10 by 10 cm. in area, situated just below the clavicle on the anterior aspect of the right chest wall. In addition, there was a hard nodular swelling on the right side of the neck, extending upward 8 cm. from behind the clavicle. The larnyx was displaced 2 cm. to the left of the midline.

The upper half of the right lung was completely replaced by a grayish-yellow, firm tumor mass, which had spread beyond the limits of the pleura. This mass was, first, adherent to the ribs and bodies of the upper third or fourth dorsal vertebræ, eroding the bodies to a slight extent; second, it had spread through the first intercostal space anteriorly, and had grown over the anterior surface of the chest wall, covering the first and second ribs and their interspaces; third, it had involved by direct continuation the lymph

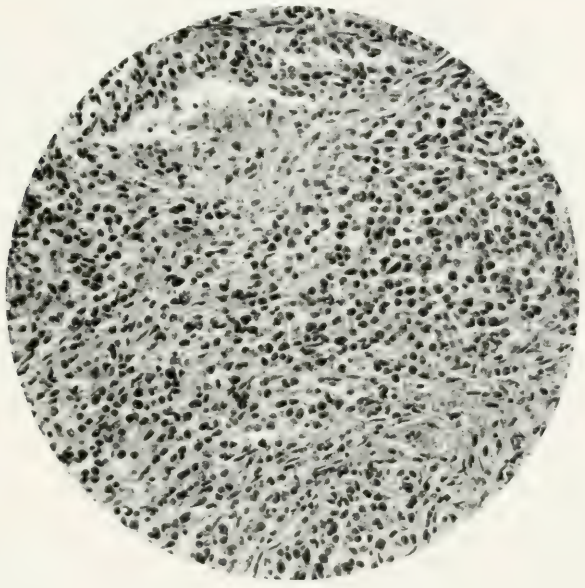

FIG. 1. Case 1. Microscopic section of primary tumor in the lung.

nodes in the anterior mediastinum, and those in the right supraclavicular region by growth behind the sternum. The pleura over the lower half of the lung was not thickened, and the lung tissue itself of these portions was atelectatic, and the seat of several hemorrhagic infarcts. Both lobes of the left lung were air-containing throughout, and on gross section presented the appearance of "brown induration."

The heart was displaced to the left, and its surface was covered with numerous petechial hemorrhages. The organ was hypertrophied and both ventricles dilated. The liver, as well as the kidneys, was congested. The

left adrenal was normal. The right adrenal, however, was replaced by a hard, discoid mass, 7 cm. in diameter and 2.5 cm. in thickness, which on gross section was uniformly white, except for a few small yellow patches.

Microscopically the tumor of the lung was seen to consist of a diffuse growth of moderately large, round and polyhedral epithelioid cells. The center of the lung tumor was extensively necrotic. In the lymph-nodes, and on the edges of the tumor, there was fibrosis, but in these regions also there were present small areas of necrosis (Fig. 1).

The same type of cell was found in the right adrenal, lying among remaining small islands of adrenal tissue (Fig. 2).

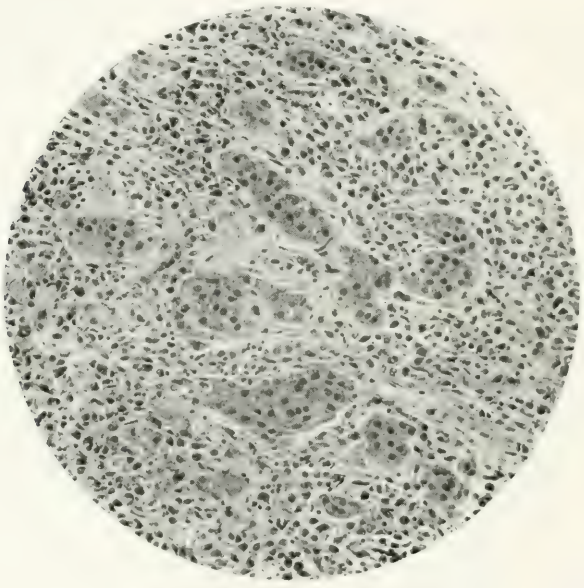

FIG. 2. Case 1. Metastatic infiltration of cells among the remaining islands of adrenal tissue.

The anatomical diagnosis was primary carcinoma of the lung, metastases in the supraclavicular and mediastinal lymph nodes, and metastasis in the right adrenal.

Second Case: The patient was a woman, 34 years old, who was admitted to the hospital on October 27, 1916, complaining of shortness of breath, weakness, loss of weight, and an ulcer in the neck just above the sternum.

In January, 1916, she had noticed some fullness in the left supraclavicular region. In February, 1916, a nodule appeared in the midline of the neck just above the sternum, which her physician incised. The wound healed, but soon broke open again, and continued to discharge a serous material.

On physical examination at her first admission (in October, 1916) there

was noticed some prominence and fullness over the upper portion of the left side of the chest, extending into the supraclavicular region. From the apex to the fourth interspace on this side there was dullness on percussion, but good resonance below to the base of the lung. The heart was displaced to the right. In the midline of the neck, just above the manubrium there was an irregular ulcer 3 by 5 cm.

During her stay in the hospital she received three *x*-ray treatments. Three weeks after her last treatment her condition was reported to be as follows: "Externally, the ulcer above the sternum is healed. The swelling in the left side of the neck is appreciably smaller, and the patient is gaining weight." Her physical condition improved right along, and by June, 1917, she had gained thirty pounds in weight. A radiographic examination of the

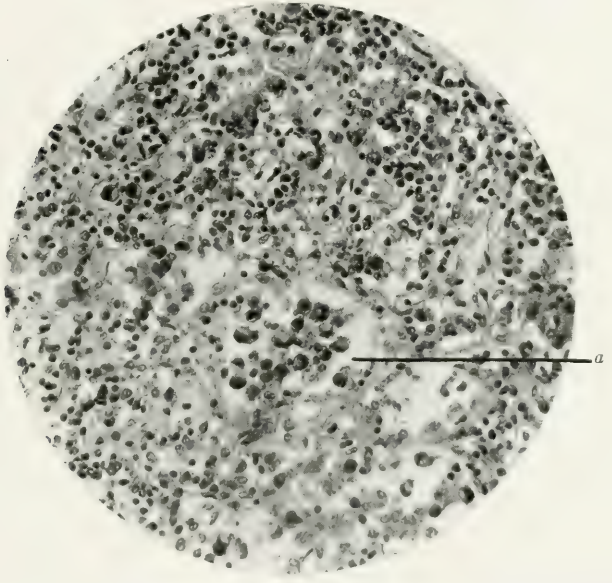

FIG. 3. Case 2. Shows at "a" a group of carcinoma cells filling an alveolus of the lung, on the edge of a small nodule of cancer.

chest (September, 1917) was reported as showing "an improvement in the chest condition."

In February, 1918 (two years after the onset of her illness), she was readmitted to the hospital complaining of considerable dyspnea. She again received radium and *x*-ray treatments.

On February 20, 1918, the patient died, having complained for two or three days before her death of numbness in her arms and feet, and of difficulty in urinating. The day before her death her legs had become completely paralyzed.

The autopsy was performed the day following her death. The skin over the chest was brown from *x*-ray treatment. The upper lobe of the left lung was converted into a solid, opaque, fibrous tumor mass, tightly adherent to the pleura, and eroding the bodies of the first dorsal and the last cervical vertebræ, and the first rib.

The lower part of the upper lobe of the left lung was the seat of a circumscribed mass, 6 cm. in diameter, composed of firm tumor tissue. It was opaque, yellowish, and apparently necrotic. All the structures in this region were extensively deformed by cicatricial connective tissue. Throughout the lower lobe were found a dozen nodules (1 to 2 cm. in diameter), one of which showed central hemorrhage and excavation. The right lung showed tumor masses occupying the lower and middle lobes. One was large, 8 cm. in diameter; one measured 4 cm.; and there were several smaller ones. The parenchyma was much congested. The pleura was the seat of corrugated fibrous exudate. The bronchial nodes on both sides were much enlarged by similar tumor tissue. The bodies of the first dorsal and the last cervical vertebræ were invaded and eroded by tumor tissue, exposing the dura, and compressing the cord at this point.

Microscopic sections showed the tumor to consist of large round and polyhedral cells, extremely anaplastic. In this case, as in the first, there was much necrosis. On the outskirts of the smaller nodules groups of tumor cells were seen lying within the alveoli of the lung (Fig. 3).

The anatomical diagnosis was primary carcinoma of the upper lobe of the left lung, metastatic tumors in the right lung, erosion of the spinal column, and compression of the cervical cord.

In contrast with the first case, in which there was no treatment and the patient lived only one year, it is worth noting that this patient improved apparently for a time after her *x*-ray treatment, and lived two years. Actual regression of the tumor growth was noted clinically.

It is generally accepted that it is almost impossible to determine the histogenesis of a fully developed primary lung tumor, and it is only rarely that a tumor so small is met with that its very beginnings can be clearly seen. Still, observations on such early tumors have shown that carcinoma of the lungs can arise from (1) bronchial epithelium; (2) bronchial mucous glands; and (3) respiratory epithelium of the alveoli.

Although it is not possible, as said, to separate all the advanced cases, still the earlier stages can, to a certain extent, be distinguished by gross anatomical and histological features.

Carcinoma of the lining epithelium of the bronchi begins usu-

ally at the hilus of the lung. In the early stages, papillary projections of the mucosa may be seen; in advanced stages, the tumor mass may surround the hilus and the large bronchi. There may be extensions to the pleura, and the chest wall may be invaded. Microscopically, the growth is usually either a squamous-cell carcinoma or a cylindrical-cell alveolar carcinoma, but other types are also quite frequent.

Bronchial carcinoma arising from the mucous glands has been seen in its early stages to be limited to the submucosa of the bronchi, with the bronchial mucosa over it remaining relatively intact. Since there is no, or only very slight ulceration of the lining mucosa, stenosis of the bronchi, rather than dilatation, is produced. In advanced cases there is infiltration of the surrounding parenchyma.

The microscopic structure is usually that of an adenocarcinoma. Mucus, which may be present within single cells, or which may fill and distend the alveoli, is usually present in considerable quantity. This mucous secretion in a primary carcinoma of the lung is strongly suggestive of mucous gland origin. Carcinoma arising from the respiratory epithelium may be either diffuse or multiple and nodular. In the diffuse form one whole lobe or the greater portion of one whole lung is consolidated. Necrosis is prominent. Owing to the uniform distribution and color of the new growth, it often resembles organizing pneumonia, or lobar pneumonia in the stage of grey hepatization. The pleura usually becomes involved.

Local and general metastases of lung carcinomata are frequent. Metastatic foci may develop in the same or opposite lung, and it is probably in this way that the nodular form of carcinoma arising from respiratory epithelium develops. One or both lungs may be the seat of multiple tumor nodules, varying in size from a miliary tubercle to nodules several centimeters or more in diameter. Necrosis is a prominent feature even in the small foci.

The histological structure of carcinoma of respiratory epithelium is variable; but it is usually made up of cuboidal, cylindrical or flat cells. The air vesicles are often filled with groups of cuboidal or polyhedral cells.

The two cases reported here represent advanced cases of pulmonary carcinoma, and it is, therefore, a matter of some difficulty to determine their point of origin. Neither of them was limited to the hilus, the usual location of carcinomata arising from bronchial mucosa, so that they are probably not of bronchial origin. Histologically, they show no trace of mucous glands or mucous secretion, which rules them out of the class of mucous gland origin. The first case involved diffusely the whole upper half of the lung, and the cells were large polyhedral cells. These are the gross and microscopical features seen in the diffuse form of carcinoma derived from respiratory epithelium. The second case presented multiple nodules scattered throughout both lungs, with the oldest lesion situated at the left apex. The cells were large, diffusely growing, polyhedral cells, with small masses of cells in the surrounding alveoli. Necrosis in both cases was extensive, and occurred even in the small nodules.

From these brief considerations of the gross and histological features, these cases can most reasonably be assigned to the group of pulmonary carcinomata, having their origin in respiratory epithelium, and representing, respectively, the diffuse form, and the multiple and nodular form of this group.

Discussion:

Dr. Lambert: I have been rather fortunate in being able to study ten primary carcinomas of the lung. Five of these occurred in the autopsy service of the Presbyterian Hospital during the past ten years, three came from the Montefiore Hospital, the remaining two were presented to me by Dr. Rohdenburg and Dr. Wood. Practically all of these ten specimens could be placed fairly easily in the several recognized groups of primary carcinomas of the lung, a little more readily, I think, than the two tumors which Dr. Muller has demonstrated. One of my cases was composed of cells showing perfect cilia in places. This is a well recognized type. In another of the tumors the cells were arranged in the form of acini, the lumina of which were filled with mucous. The origin of the tumor was clearly in the mucous glands of the bronchi. Four of the tumors belonged to the squamous cell group, which it is generally agreed, are derived from bronchial epithelium. The cells in the other tumors were not so well differentiated. I thought that their origin was probably from the epithelium lining the alveoli. I was rather struck by the fact that the tumor composed of the ciliated epithelium, that is, of cells which had departed least from the normal type, appeared to

be most malignant, with widespread metastases including multiple nodules in the brain. Metastases in the central nervous system were found in several cases, and I believe it was long ago observed that lung tumors often exhibit this tendency. One of the tumors was exceedingly small, only 2 or 3 cm. in diameter.

Dr. Muller: In these cases we usually found two types: either it involved a large portion of the lung, or we found nodules which arose from a primary focus. I think these nodules are probably metastatic nodules, I mean arising from the cells carried either by the lymphatics or the blood stream. My idea was that they all arise from some primary focus, but we found at autopsy one of these conditions: either the carcinoma involved a large portion of one lung, or it is in the form of a number of nodules.

Dr. Moschcowitz: Clinically these tumors have interested me greatly. Often their only symptom is dyspnea, with or without bloody expectoration. My own experience is that expectoration is not very common in the early stages of lung carcinoma. I believe that a good many "mediastinal" tumors are really cases of primary bronchial carcinoma. I recall two cases distinctly in which their origin was shown at autopsy. A considerable number of these primary lung tumors, especially in the later stages, show signs of effusion.

Dr. Lambert: I should like to ask Dr. Moschcowitz if he has seen in his wide autopsy experience any of the so-called endothelioma of the lung. I have never been fortunate enough to find one, and have had doubts as to whether any such tumor really existed. Clinically at least half of the cases in my series had been diagnosed endothelioma, but as I have stated, all of them proved to be definite carcinomas. Recently I performed an autopsy on a case where there was a large sarcoma of the lung, the only one I have ever seen.

Dr. Moschcowitz: I have a number of the so-called endotheliomas of the pleura. There is a coating of the pleura half an inch thick. The lung itself is not involved in the slightest degree. My own opinion is that they are not endotheliomas, but carcinomas.

CONGENITAL NEUROBLASTOMA OF NOSE

ELI MOSCHCOWITZ, M.D.

The specimen was removed from a child, four months old. The tumor was present at birth, and had increased in size. It was hard, irregular, about the size of a marble, covered by skin, and situated in the median line at the root of the nose. Grossly, the tumor was hard, white, and presented a fibrous smooth surface on section; there was no capsule. Microscopically, the

tumor consisted of nerve and fibrous tissue. The nerve tissue was composed of irregular strands of non-medullated nerve fibers in which were scattered numerous ganglion cells, which were round or oval, with round central nuclei, and occasional nucleoli. These strands were sharply defined from the surrounding connective tissue which formed an interlacing network between the strands of nerve tissue. The connective tissue was densely fibrous. The tumor extended from the subepithelial layer of the skin to the depths of the section where it became lost.

As far as study of the literature shows, this is the first congenital neuroblastoma (or ganglion neuroma) on record. The majority of these tumors occur in early childhood; Thomson in his monograph says that they have been reported up to the age of thirty years, but Hanel reports a case in an individual of forty-six. They may be single or multiple, circumscribed or diffuse, and vary in size from a pea to a child's head. Despite their nerve origin, they cause no sensory disturbance. Their consistency varies from that of a lipoma to a myoma. The tumors, as a rule, are not capsulated; they are, nevertheless, only rarely malignant, the only malignant cases reported being those of Beneke and Miller. These tumors are supposed to arise from the sympathetic system, the main points of evidence being the lack of myeline sheaths and their common occurrence along the main stems of the sympathetic nervous system. These tumors have a predilection to occur along either side of the vertebral column, in the neck, chest, and abdomen. They also occur in the cerebrospinal system (Cerny, Benda), in the subcutaneous tissue (vascular nerve plexus) (Knauss, Kredel-Beneke), in the medulla of the adrenal (Miller), in the capsule of the knee (Hagenbach), the dura, the alæ of the nose and vagina (Beneke). In twenty-six cases, Courvoisier found only four with myeline sheaths.

Discussion:

Dr. Lambert: I have never seen an example of the tumor which Dr. Moschcowitz has described, but from his description I should say that it ought not to be called "neuroblastoma," since by general agreement in recent

years this term has been applied to a malignant growth arising from the undifferentiated cells of the sympathetic system, a tumor located most often in the adrenals and showing a tendency to metastasis. Dr. Moschcowitz's tumor is composed, I understand, of well differentiated nerve cells and nerve fibers, and it seems to me it should be called a "ganglio-neuroma." Unfortunately, there is no body with power to settle these questions of nomenclature, but when a name has become associated with a type of tumor, confusion would be avoided, if the term were not used for any other neoplasm.

Dr. Moschcowitz: The majority of writers call it neuroblastoma. Kaufmann calls the variety of tumors referred to by Dr. Lambert neurocytoma.

THE VALUE OF THE WASSERMANN REACTION: A REPLY

JOHN H. LARKIN, M.D., I. J. LEVY, M.D., AND JOHN A. FORDYCE, M.D.

(From Pathological Laboratories, City Hospital, New York)

A critical analysis was made of a paper by Symmers, Darlington, and Bittman in which an attempt to correlate the necropsy findings with the results of the Wassermann test during the life of the patient resulted in the conclusion that the Wassermann reaction is not dependable in the diagnosis of syphilitic infections, and of little value as an indication of the cure of the individual affected with syphilis. Dr. Larkin and his co-workers point out that the Wassermann test includes several methods of serologic procedure and that an accurate interpretation of each method is essential in arriving at a proper diagnosis. The following conclusions are reached:

"A positive reaction is the most constant symptom of syphilis.

"The value of the reaction in diagnosing undoubted syphilis is shown by the fact that:

"1. The reaction is positive in practically 100 per cent. of the cases of florid syphilis.

"2. In active tertiary syphilis of the skin and bones the reaction is positive in about 94 per cent. of the cases.

"3. In syphilis of the central nervous system, cognizance must be taken of the reaction in both blood and spinal fluid. The blood is positive in about 80 per cent. of the cases.

"4. In a pathological study, the Wassermann reaction (alcoholic antigen, warm fixation) was positive in 94 per cent. of cases of syphilitic aortitis.

"As a means of corroborating syphilitic infection, the Wassermann test is at least 90 per cent. dependable, as shown in a series of positive reactions in which 90 per cent. could be accounted for by syphilitic changes in the aorta alone.

"The value of a negative reaction has been studied and its reliability confirmed by the negative reactions obtained in non-syphilitic affections of the skin. In a series of necropsies in which it was demonstrated pathologically that the aorta was free from syphilitic disease, negative reactions were obtained in 91 per cent.

"The conclusions of Dr. Symmers and his co-workers are shown to be fallacious, owing to:

"1. The apparent disregard of the different results obtained by various serologic methods and the employment of a questionable technic.

"2. The careless survey of pathological material."

(The paper is printed in full in the *Journal of the American Medical Association*, 1918, lxx, 1589.)

THE VALUE OF THE WASSERMANN REACTION: A CORRELATION OF THE SEROLOGICAL AND POST-MORTEM FINDINGS AT THE PRESBYTERIAN HOSPITAL, NEW YORK

ROBERT A. LAMBERT, M.D., MIRIAM P. OLMSTEAD, AND HAROLD C. STUART, M.D.

The Wassermann reaction as a test for syphilis has been in general use for more than ten years, but there is still considerable difference of opinion, especially among clinicians, as to its diagnostic value. It is generally agreed that a majority of syphilitics, especially in the early cases, give a positive test. But there is a lack of agreement as regards, first, the percentage of cases of syphilis giving a negative reaction, and, secondly, the frequency of positive reactions in non-luetic cases. It is obvious that the diagnostic value of the test may be accurately measured by the answers to the questions implied in the two points just stated.

It is hardly necessary to mention the fact that numerous reports bearing upon the value of the test have been published in recent years. In most of the reports, however, the serological findings have been controlled only by the clinical history and physical examination of the cases, in which there is a well recognized wide margin of error. There can be but little question that the only reasonably accurate method of checking up the Wassermann test lies in a complete gross and microscopic examination of the body after death.

This method of estimating the value of the Wassermann reaction has been applied at Bellevue Hospital by Symmers and his co-workers, who have recently published a report, the conclusions of which are as follows:

"1. Depending on the antigen employed, the Wassermann reaction in the living patient, as carried out at Bellevue Hospital, gives a negative result in from 31 to 56 per cent. of cases in which the characteristic anatomic signs of syphilis are demonstrable at necropsy.

"2. The Wassermann reaction in the living patient is positive in at least 30 per cent. of cases in which it is not possible to demonstrate any of the anatomic lesions of syphilis at necropsy."

Since these results, if substantiated, would make questionable the wisdom of the present general use of the Wassermann test, it seemed to us important that more data bearing on the question should be collected. In pursuance of this object we have attempted a correlation of the serological and autopsy findings in cases coming to autopsy at the Presbyterian Hospital during the past three years. During this period a uniform method of carrying out the Wassermann test has been followed, and complete records of all autopsies have been kept, including gross and microscopic descriptions of every organ.

As the Wassermann technique has been subjected to various modifications, it may be well to give in some detail the essentials of the method in use at the Presbyterian Hospital.

TECHNIQUE

Two antigens are used in all tests: a crude alcoholic extract of guinea-pig heart, and a cholesterinized extract of guinea-pig heart. The ice-box fixation method for four hours is employed with the alcoholic antigen; water bath fixation at 37° for one half hour with the cholesterinized antigen. Serum is inactivated one half hour at 55° C. The sheep system is used. Complement from each guinea-pig is titrated separately, against one and one half units of amboceptor, and is so diluted that a dose of one and one half units is used in the alcoholic tests, and a dose of two units in the cholesterin tests. Each specimen of complement is also tested with varying amounts of the cholesterin antigen, the amount to be used in the tests, twice, three times, and four times that amount. Any specimen that fails to hemolyze completely in the presence of the cholesterin antigen is not used in the cholesterin tests, but it may be used with the alcoholic antigen, if its hemolyzing power is good, according to the complement titration. An amboceptor titration is made with each mixture of complement, that for the alcoholic tests and that for the choles-

terin; and for sensitizing the cells, the amboceptor is so diluted that one dose contains two and one half units. Tests are read as soon as hemolysis is complete in the antigen and serum controls, in which there is double the amount of antigen and serum used in the tests. By a ++++ reaction is meant complete inhibition of hemolysis in 0.2 c.c. of serum. Degrees of incomplete inhibition are indicated roughly by +++, ++, and +. Only a ++++ is considered positive.

The results of the serological and autopsy findings in the 214 cases analyzed can be best presented in the form of tables. On the basis of the post-mortem findings the cases are divided into three groups: (1) Syphilitic, or "positive" cases; (2) non-syphilitic, or "negative" cases, and (3) "doubtful" cases.

TABLE I

Autopsy Findings	No. Cases	Wassermann			
		++++ (Both Antigens)	0 (Both Antigens)	+, ++, +++ (With Either Antigen)	
Positive	23	18	3	2	= 85.7 % Positive (Excluding 2 Treated Cases Giving Doubtful Reactions)
Negative	188	0	175	13	
Doubtful	3	2	0	1	
Total	214	20	178	16	

TABLE II

Cases Showing Positive Autopsy Findings

	No. Cases	Wassermann		
		++++	0	?+, ++, or +++ (With Either Antigen)
Syphilis of aorta and heart	15	11	2	2 (treated cases)
Tabes	1		1	
Congenital syphilis	2	2		
Miscellaneous	5	5		
Total	23	18	3	2

Table I is designed to show at a glance the results of the Wassermann test in these three groups. It is seen that 18 out of 21 cases of proved untreated syphilis had given during life a

++++ Wassermann with both antigens. A majority of these cases were examples of late syphilis, as indicated in Table IV. The most significant fact brought out in this analysis is that of 188 cases showing no evidence whatever of syphilis at autopsy, not one had given a ++++ reaction. Three cases were doubtful anatomically. In two of these, with very suspicious lesions in liver and testis, there was a ++++ Wassermann. In the third, a chronic orchitis, the reaction was negative.

TABLE III

Wassermann	No. Cases	Autopsy Findings		
		Positive	Negative	Doubtful
++++ (Both antigens)	20	18	0	2
0 (Both antigens)	178	3	175	0
+, ++, +++ (With either antigen)	16	2	13	1
Total	214	23	188	3

In Table III the data are so arranged as to bring out the significance of the Wassermann reaction in the light of post-mortem findings. In other words, the figures constitute the pathologists' answer to the question as to what a positive, negative, or doubtful Wassermann reaction means. The table shows that 20 cases gave a ++++ Wassermann test with both antigens and that in 18 of these characteristic syphilitic lesions were found at autopsy. One of the two remaining cases showed suspicious lesions in the liver, very probably luetic in origin, while in the other there was a chronic orchitis, possibly syphilitic. It is thus seen that 18 out of 20, or 90 per cent. of the positive reactions, were conclusively confirmed by autopsy findings and that in the two remaining cases there were lesions strongly suggestive of syphilis, a possible margin of 10 per cent. Of 178 patients giving negative reactions with both antigens, only three showed definite syphilitic lesions at autopsy. That is, the negative Wassermanns were confirmed in approximately 98 per cent. of the cases—a margin of error of only 2 per cent.

There were 16 cases giving reactions classified as doubtful (+,

++, +++) with one or both antigens. Two of these were cases of treated syphilis; one showed lesions in meninges, testis, and liver, possibly syphilitic in origin. In the 13 remaining cases no anatomical evidence of syphilis was found. Excluding the two doubtful reactions in treated cases, 13 out of 14, or 92.8 per cent., of the doubtful Wassermanns were non-specific as judged by autopsy findings. It is a noteworthy and possibly significant fact, that 11 out of 16, or 68.7 per cent., of these doubtful reactions were given by the cholesterin antigen only. In only one instance was there a doubtful reaction with the alcoholic antigen alone. It is thus evident that while the cholesterin antigen may have the advantage of giving more reactions in cases of syphilis, and of more nearly excluding syphilis, it has the disadvantage of giving a considerably larger number of apparently non-specific reactions, as judged by autopsy findings.

TABLE IV

Doubtful Reactions

No. Cases	Antigen	
	Alc.	Cholest
11	0	?
1	?	0
4	?	?

Total 16 ?=+, ++, or +++

A review of the data presented above shows a close parallelism between the serological and autopsy findings at the Presbyterian Hospital during the past three years. The results are at variance with those obtained by Symmers and his co-workers at Bellevue Hospital. The reasons for this difference are not very evident; though they are probably due, in part at least, to differences in the technique of carrying out the test. Such a wide discrepancy in the results obtained at two large institutions where both the serological and pathological examinations should be well done, would indicate the need for further data on the Wassermann question.

CONCLUSIONS

A comparison of the serological and autopsy findings at the Presbyterian Hospital during the past three years brings out the following facts:

1. Among cases showing anatomical evidence of syphilis at autopsy 85.7 per cent. had given during life a ++++ Wassermann with both alcoholic and cholesterinized antigens.

2. Among 188 cases showing at autopsy no anatomical evidence of syphilis, 93 per cent. had given during life a negative Wassermann with both antigens and 7 per cent. doubtful reactions (+, ++, +++ with either antigen).

These findings, in our opinion, warrant the conclusion that the Wassermann test, properly carried out, should be of great aid in the diagnosis of syphilis.

Discussion:

Dr. Norris: I do not think that I care to discuss this matter. I distinctly recognize the value of these papers, and they are quite in keeping with a number of papers published before. The subject does not lend itself easily to discussion and since there has been more or less of an individual attack in the first paper I do not think it would seem suitable on my part to make a reply to a reply. There are a certain number of unwarranted assumptions in the paper of Dr. Larkin. In the first place he seems to assume that Dr. Symmers did all the serological tests himself. He did not do them at all. They were made either by Dr. Field or Dr. St. George, or under their direction. The methods that were used are known as Dr. Field's; certainly his publications are of some value, and Dr. Symmers' attempt was merely to correlate the gross findings with the results obtained in general laboratory routine work. I do not care to uphold the dignity of Bellevue, because I do not think it necessary. About three or four years ago in doing routine pathological autopsy work I came across a number of cases in which there were fifteen or eighteen with positive Wassermanns, and the first two of them opened my eyes thoroughly. At that time I think Dr. Field was doing only the cholesterin test. So far as we could tell at autopsy there was no evidence of specific infection; there had been no treatment, and there was no history of syphilis. When you run across a certain number of such cases they disconcert you. However, I see no reason for further discussion. Dr. Symmers is amply and fully able to take care of himself.

Dr. Ottenberg: I also do not feel very much like entering into a discussion, as apparently there is something of the personal element involved. I will say this, that until Dr. Norris spoke I felt that there was nothing further

to say because it seemed to me that the two papers which had been read settled the subject most conclusively. I still feel that, too. When Dr. Symmers' paper appeared, like everyone else who was familiar with the subject, I thought that if he was right, I must have been deceiving myself very greatly in over 20,000 Wassermanns I have personally done in which I have investigated the clinical histories and wherever I have been able to, have followed the case to autopsy. As a matter of fact, after reading the paper more closely, it answers itself. I have no personal bias in the matter at all, but in going over the figures, the number of inconsistencies between the tests with simple alcoholic and those with cholesterin reinforced antigens was so enormous that one or the other methods must be at fault. Now I know that in the use of the cholesterin antigen Dr. Field's method had been followed at Bellevue. In testing out methods used in various institutions in New York City a few years ago I tried out that method. and the records showed a larger percentage of errors with Dr. Field's method than with any other. However, I did not conclude that the method was to be rejected at that time, but held judgment in reserve, because I had only used it in some two hundred cases. Later I gave it up in order to try other methods, and finally used the Walker and Swift one as the most reliable with the cholesterin antigen. I finally recognized, as Miss Olmstead says, that there is a certain number of non-specific results with cholesterinated antigen, but it seems to me that with this knowledge, and with the knowledge of the source of these errors on one side or another, it is possible to explain what has occurred.

Dr. Ewing: I agree with Dr. Norris that the subject of the Wassermann reaction does not lend itself well to discussion. There are so many intricate questions involved on both sides that the matter can hardly be settled by any form of debate. If there is any personal bias in the papers presented, this is doubtless only a sign of the healthy interest that is quite generally taken in the merits and demerits of the Wassermann reaction. I also think that Dr. Symmers' contribution will take care of itself and I have no intention of attempting its defense.

It seems to me, however, that it is very difficult to make any significant comparison of the results of the Wassermann reaction and the anatomical signs of syphilis in the body, because there are so many variable factors in both fields. Consider, for instance, the uncertainties regarding the technique of the Wassermann reaction. Not long ago several laboratories in New York consulted together and endeavored to establish a standard technique. The results were so divergent that the conclusions were never published, and there has been continuous recrimination between technicians regarding the different methods adopted. Anyone listening to Miss Olmstead's exhaustive presentation will realize how complex and delicate a thing this Wassermann technique is.

On the other side it must be recognized that the subject of the anatomical signs of syphilis is a very large one. There is hardly anything that requires more experience on the part of the pathologist than the identification of the lesser lesions of syphilis by gross and microscopical tissue changes. I think it quite likely that any two pathologists might differ regarding certain pos-

sibly syphilitic lesions, unless the *Spirochæta pallida* were demonstrated in the lesion. In the demonstration of spirochetæ we have the only positive sign of syphilis, but these organisms are often very difficult to find and may be absent in old syphilitic scars. Nevertheless, the organs of subjects who had had well marked syphilis during life regularly show rather definite lesions, the character and significance of which have been established by the older pathologists by much labor extending over many years.

The great weakness of the Wassermann reaction is that it is not a specific reaction. What possible essential relation can there be between an alcoholic extract of heart muscle of a guinea-pig and the bodies of *Spirochæta pallida?* I do not think that serologists have paid enough attention to the exact meaning of this reaction, which seems to indicate only the presence in the blood of an antigen reacting against derivatives of red blood-cells or other chemically identical substances. Based on this non-specific test, clinical medicine, chiefly in the United States, has gone to great lengths, and even committed some absurd blunders. One of our enterprising Western States decided that it was necessary to have a negative Wassermann reaction before anyone could have a marriage license, and they passed a law to that effect. But the law was soon pronounced unconstitutional by their Supreme Court; it was found that the laboratories of the State could not carry out the work, and it is also clear that the law is medically unsound. In several diseases, notably in chronic gastritis, clinicians have attempted to establish a syphilitic etiology on the basis of the Wassermann reaction. Now there is no doubt that this reaction is of value in enabling clinicians to take care of the sick, but when it comes to establishing new syphilitic diseases by means of such evidence, it is time to protest. Even as a clinical test it has seemed to me to have very definite limitations which are seldom emphasized by its sponsors, and I have often been deeply impressed by the errors into which experienced clinicians have been led by too exclusive reliance on the test. Moreover, it is just in these doubtful cases which have appeared in one of the papers this evening, that one wants help from the Wassermann; and it is just in such cases that the Wassermann fails.

There is quite a long list of diseases in which the Wassermann reaction occurs frequently, including malaria and other acute infections, tuberculosis, leprosy, jaundice, pernicious anemia, hemoglobinemia, and even acidosis.

I am inclined to think that the limitations of the Wassermann reaction are being recognized more and more by conservative clinicians and laboratory men, and I know of several who speak with much less confidence about the test now than they did some years ago.

Dr. Moschcowitz: I wish to make a few remarks from the viewpoint of the clinician. I believe a distinction should be made between interpreting the value of the Wassermann reaction from the standpoint of the pathologist and from the standpoint of clinical findings. Certainly in the wards of a hospital and in general practice the Wassermann reaction bears out clinical expectations very closely. In other words, a positive Wassermann reaction is only exceptionally found when it has not been expected; the converse is equally true.

Dr. Larkin: As one of the contributors to this paper and as a member of the Pathological Society I wish to say that in the presentation of this subject no personal bias has tinctured our remarks or influenced our conclusions. Now I think it is the attitude of our scientific men, and it has been the attitude of the Pathological Society, to have an impersonal and unbiased discussion of any scientific subject, and I feel that my colleague, Dr. Norris, has taken an extremely unjust attitude, which I highly resent, in his remarks on our presentation. We have all met here many a time in a "healthy" way for discussion, and our ideas should be exchanged without indulging in any personalities. In preparing this paper it has been the ambition of the authors to eliminate the personal side. I cannot discuss at length the figures which have been gone over because there are other papers to be presented here. I only wish to say that one or two of Dr. Ewing's remarks might bear some discussion. One of them is his observation on the Wassermann reaction in tuberculosis. It has been my endeavor to study personally the character of the reaction in pretty nearly 20,000 cases of tuberculosis at an early stage which have been very carefully gone over. This is a task requiring much labor, and perhaps when we have more knowledge of the Wassermann reaction in syphilis we may be able to present some "healthy" discussion on the subject. Of course, Dr. Ewing is something of an iconoclast, and it is hard to convince him. One remark is noteworthy, and that is the attitude of the surgeon in regard to syphilis. From a very large experience I have seen only two cases of gastric syphilis, though I have seen a number of cases which have been sent to me, but which showed no evidence of the lesion in the gross or microscopically, despite the fact that the clinician and the surgeon very likely made the assertion that the individuals had syphilis.

TWO CASES OF INTESTINAL ANTHRAX WITH SMALL SKIN LESIONS AND EXTENSIVE MENINGEAL HEMORRHAGES

A. ZINGHER, M.D.

The first patient was seen about the middle of November and was admitted to the Base Hospital in a very delirious condition, from which he passed into a state of coma. The patient had convulsions, and died on the evening of the day of admission. No special history could be obtained that the patient had handled infected meat, infected horsehides, or that he had used a new shaving brush. A lumbar puncture was made soon after admission to the hospital, and the spinal fluid was uniformly bloody.

Microscopical examination showed an increased number of polynuclear leucocytes, many red blood-cells, and a very large number of anthrax bacilli arranged in chains and branching forms, and showing irregularities on staining. Subsequent cultures showed a typical anthrax growth, and the organism produced a typical fatal infection in the guinea-pig. The finding of this organism in the spinal fluid drew our attention to a rather curious lesion of the skin on the right side of the neck. The lesion measured 1 by 3 cm., was indurated, and covered in the center by a firmly adherent superficial scab. The color of the lesion was coppery in hue. The lesion was surrounded by a lighter colored areola. There were also scattered petechiæ and ecchymoses on the skin.

The autopsy showed a very interesting condition. On opening the abdomen an extensive edema of the retroperitoneal tissues was noted. Occupying the large intestine at the hepatic flexure was a mass the size of a fist. On opening the intestine at this point a very extensive edema of the mucosa was noted, extending for about eight inches into the ascending and transverse colons. The center of this edematous mucous membrane was occupied by a small ulcer the size of a twenty-five cent piece covered by a superficial area of necrosis. The rest of the small and large intestine showed no other lesions. The liver and kidneys showed parenchymatous degeneration. On removing the skull cap an extensive hemorrhage covering both cerebral hemispheres was noted. The hemorrhage was sufficient to obscure the underlying convolutions of the brain. This hemorrhage was within the pia-arachnoid and was found to extend into the sulci dipping in between the convolutions of the brain. The blood culture taken ante-mortem showed the anthrax bacillus.

The second case was admitted to the Base Hospital about the middle of April, 1918. The patient had been treated two days before admission to the hospital for an ingrowing hair in the right side of the neck anteriorly. The abscess of the neck increased in size, causing the patient to be sent to the Base Hospital. Two days later an edematous hemorrhagic area developed

over the right buttock. No definite diagnosis was made. On the fourth day after admission to the hospital the patient complained of sudden dizziness, weakness, and showed slight general convulsions. He went into coma and died three hours later.

At the autopsy an intense edema of the retroperitoneal tissues was noted, with circumscribed hemorrhagic areas, varying in size from a ten- to a fifty-cent piece, scattered along the entire course of the large and small intestine. These lesions, especially the intense edema, made us suspect anthrax. The edema along the ascending colon was so extensive that the large intestine was pushed forward and inward. There were about three liters of slightly turbid fluid in the peritoneum, the surface of the peritoneum showing an acute congestion. On opening the intestine there was found corresponding to each area of hemorrhage a lesion of the mucous membrane. Over this lesion the folds of mucous membrane were rigid and showed hemorrhagic infiltration. Superficial erosions were present on these folds, and were covered with a thin necrotic membrane. The lesions were more extensive in the large intestine, and here the rest of the mucous membrane was also considerably edematous. The brain showed the same hemorrhagic condition in the pia-arachnoid as was noted in the first case. The lesion on the neck was slightly undermined, and the pus showed Gram-positive cocci, as well as anthrax bacilli.

The bacteriological findings in this case were interesting. Anthrax bacilli were found in the ventricular fluid of the brain, in the lesion of the neck, and in the peritoneal fluid. The blood showed a bacillus post-mortem. The peritoneal fluid also showed a Gram-positive coccus in addition to the anthrax bacillus.

In conclusion, I may state that both the cases of anthrax which were observed and autopsied showed anthrax skin lesions, extensive meningeal hemorrhages, and intestinal lesions, the first case showing a single lesion of the large intestine at the hepatic flexure, the second case multiple lesions of the small and large intestine. The anthrax bacillus was recovered in both cases from the cerebrospinal fluid, blood, and local lesions. No history

was obtainable of exposure to the infection. Clinically such cases might be mistaken for different forms of meningitis, but a lumbar puncture should exclude such a diagnosis.

STREPTOTHRIX INFECTION OF LUNG

E. FINCH, M.D., AND D. S. D. JESSUP, M.D.

(From the Pathological Department of the New York Skin and Cancer Hospital)

The interest attached to this case of streptothrix infection lies in the fact that the organism was found by blood culture three days before death, and that at autopsy it was present in a large tumor of the lung which in the gross in every way suggested a malignant new growth, but which microscopically gave the histological picture of a granuloma.

The patient, a woman of 35 years, was under observation in the wards of the hospital for about eight weeks, in the service of Dr. George H. Semken, through whose courtesy the case is presented. There were a distressing cough and intermittent attacks of fever, and radiograms showed lung involvement. The sputum was more or less tenacious and yellow, and contained minute yellowish bodies, which on crushing showed masses of pus and endothelial cells with large numbers of bacteria and an occasional mycelial thread. The clinical diagnosis was tuberculosis but repeated examinations of the sputum failed to show any tubercle bacilli and a von Pirquet test was negative. Actinomycosis was thought of, but no ray fungi could be found.

Blood Culture.—This was made three days before death. On the next day there were small colonies of mycelium just above the layer of blood in the bottom of the flasks of broth and the blood was becoming black. Small colonies were picked and lifted out easily in their entirety and transferred to blood agar and to Loeffler media and grown at body temperature. On these two media growth appeared on the second day as convex colonies

and on the sixth day as confluent colonies from 3 to 4 mm. in size, leathery and tough, and breakable only with a stiff loop. There was no efflorescence on any of these colonies. Those on the blood media were dark, almost black in color, with a change in the media to black. Those on the Loeffler media were about the color of the media. Microscopically the broth colonies showed Gram-positive, intertwined masses of long, delicate, branching threads with a few spores about the size of staphylococci. There were no clubbed extremities. Teased fragments from the convex colonies from the solid media showed the same threads in denser masses. All the cultures, but especially those on the solid media, possessed a strong mouldy odor. The organism was identified as the *Streptothrix pulmonalis* and closely resembled the *Streptothrix hominis*, type III of Foulerton. At the time that the blood culture was made the sputum was again examined and a large number of very small plugs found which showed tangled masses of mycelia mixed with pus cells and bacteria.

Cultures of the organism kept for six months on solid media in the ice-box show a less convex surface and at the borders of the growth a very slight efflorescence. The mycelial threads were easily broken up with an ordinary loop.

Autopsy.—Permission for a partial autopsy only could be obtained. Examination showed a fairly well nourished woman with a small frame. There was a healed scar of a thyroid operation and a sinus on the right side (site of an operation performed during stay in hospital, when tissue had been removed on the supposition that it was thyroid). This sinus led down to a firm mass 2 cm. in size. The left pleural cavity was filled with a large amount of slightly turbid serum. The lung was compressed by this and anteriorly was continuous with a hard fibrous mass, which extended down from the sternal notch to lie over the pericardium. This measured 20 cm. in length and was 10 by 8 cm. laterally. On section it was pale, fibrous, and almost cartilaginous in spots with a few soft yellow areas. It extended backward into the mediastinum, embracing the roots of the larger bronchi and having embedded in it many enlarged bron-

chial nodes. The left lung in the portion not involved in the growth showed a few small areas that resembled consolidation and was firmly adherent to the parietal wall so that it tore out in shreds. The right lung showed fresh lymph exudate at the base. The visceral pericardium was normal, the parietal very much thickened. The heart was normal. The spleen was large and soft.

Anatomical diagnosis was tumor of lung, mediastinum and left pleura; fibrinous pleurisy; chronic pericarditis.

Microscopical examination of autopsy material: The tumor of the lung resembled most a chronic inflammatory process, some portions showing very dense connective tissue growth, and others showing a cellular infiltration, both mononuclear and polynuclear. Employing Mallory's special phosphotungstic-hematoxylin stain the blood vessels were found in many places to be plugged with intertwined masses of branching short threads with a few dark staining bodies the size of staphylococci. The bronchioles could also be seen containing the organism, and it could be found as small tangled short threads free in the tissues at some distance from the blood-vessels. It could also be demonstrated in the bronchial nodes and in large masses in the pleural exudate at the base of the right lung. In the spleen there were a few scattered threads which could be identified as mycelial threads.

Examination of the tissue which had been removed by operation some weeks before death showed with the phosphotungstic stain the mycelial threads which had been overlooked with ordinary stains at the first examination. This seemed to demonstrate that the streptothrix infection had been of long standing.

The literature shows many reports of streptothrix infections and a number of them as involving the lung. The distinct tumor formation in this case led us to suppose that we were dealing with a malignant new growth, possibly a thymus tumor. When we first examined it microscopically we thought of Hodgkin's. The faint staining of the mycelial threads by hematoxylin caused them to be overlooked but with the deep stain of the phosphotungstic method there was no longer doubt in our minds that we

were dealing with the same organisms that we had found in the blood and that the whole course of the disease had been dependent on this infection with the *Streptothrix pulmonis.*

Discussion:

Dr. Ewing: I have never recognized a thing of this character. The sections that I saw under the microscope are stained in a way that I am not familiar with. I am a pretty poor technician and find it hard to examine sections when I am not at home with the staining. But the whole picture is that of a chronic granuloma of a peculiar type. I should like very much to have some sections to study carefully, and also to try to find out the relation of the streptothrix to the lung lesion. I have no doubt that Dr. Jessup's interpretation is correct.

Dr. Larkin: I feel somewhat the same way as Dr. Ewing does about the microscopical appearance of the lungs. In 1909, Dr. Norris and myself published two cases, but the specimens we had were quite different from the one presented to-night. I do not think there is any question about the early involvement of the lung, which begins in bronchopneumonia, and it is very often associated in the early stages of the disease leading on to a diffuse granulomatous condition; but the extent of the lesion here is so remarkable that I cannot for a moment think it represents the same type of lesion before described. I should also like to ask Dr. Jessup for some of the tissue, and as I think I still have in my laboratory the two specimens to which I referred, I should be glad to give him, as well as Dr. Ewing, some of that material.

Dr. Ottenberg: May I ask if the streptothrices were found in the spleen or any of the lymph nodes? The reason I ask is because I remember reading of cases of splenomegaly in which they were found in these situations (A. G. Gibson, *Quarterly Journal of Medicine,* January, 1914, p. 153).

Dr. Jessup: In regard to their presence elsewhere, we found them in some of the bronchial nodes and in the lung at a distance from the main tumor. My idea in presenting this case was to get some discussion and other opinions, as this is rather an unusual case. I want to say that the credit for the original bacteriological study and for the tissue work is due to my colleague, Dr. Finch, who cannot be here to-night.

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SOME OBSERVATIONS ON THE RECENT EPIDEMIC OF INFLUENZA

G. L. ROHDENBURG, M.D.

(From the Pathological Laboratories of the Lincoln and Lenox Hill Hospitals, New York)

During the recent epidemic of influenza a relatively large number of cases of the disease were admitted to the two hospitals with which the writer is connected, and a comparison of the experiences in the two institutions is of interest from several standpoints. Irrespective of how the infection reached this city, the development of the epidemic in the Borough of the Bronx is well shown by the admissions to the Lincoln Hospital. Within the ambulance district of the hospital there are several areas which are comparable as far as density of population, character of the housing, and types of people are concerned. One such area embraces the upper part of the ambulance district, while another one is to be found at the southern end of the district. From September 20 to October 4 at midnight, a period of fourteen days, there were 256 admissions with the diagnosis of influenza or in-

fluenzal pneumonia. Of these admissions 72 per cent. came from the congested southern district. In other words, up to October 4, the infection was localized; after that date it became widespread. A further proof of the infection by contact is given by our records of five families, consisting respectively of three, three, five, five, and seven members. In these families particularly virulent infections were the rule, and all infected died.

A relative degree of natural racial immunity is shown by the fact that amongst ninety white nurses at the Lenox Hill Hospital, thirty cases occurred, six being dangerously ill with pneumonia, and one dying, while at the Lincoln Hospital eighty-nine negro nurses of the same age were on duty, ten of whom became infected. All of these cases were mild, save two, one of whom died.

Further numerical data based on 994 cases admitted to the Lincoln Hospital between October 1 and November 1 is of interest in showing the sex and age incidence. Thus of these cases 520 were males, and 474 females, the sex being about equally divided. The age incidence showed 263 in the first decade, 185 in the second decade, 250 in the third decade, 237 in the fourth, 48 in the fifth, and 11 older than the sixth decade of life. Of these cases 148 were transferred to other institutions owing to lack of room, and the termination of these cases has not been ascertained, though all were very mild infections when transferred. Of the cases retained at the hospital over 50 per cent. developed or were admitted with pneumonia, the exact figures being 433 pneumonias and 413 influenzas. Our pneumonia mortality was 66 per cent. Fourteen per cent. died after being sick twelve hours, an additional 14 per cent. died in the next twelve hours, an additional 5 per cent. in the next twelve hours, and 12 per cent. more in the following twelve hours, while 34 per cent. died from the fourth to the fifteenth day.

At the Lenox Hill Hospital there were admitted during the same period covered by the Lincoln Hospital figures just quoted 242 cases, of which 117 were pneumonias, with a pneumonia mortality of 54 per cent.

Our experience in the bacteriology of the disease may be de-

scribed under several heads: blood cultures, sputum examinations, post-mortem cultures, and the demonstration of agglutinins and precipitins. A total of forty-five blood cultures was made, and of these but one gave a positive result, the organism being the pneumococcus. Of 198 sputum examinations by culture and smear, *B. tuberculosis* was found once; pneumococcus five times; *B. influenzæ* seven times, and a streptococcus one hundred and thirty-six times. Of the pneumococci one was of Group I; the remainder were Group II. The streptococcus encountered was of two types: one was a diplostreptococcus which sometimes hemolyzed blood, and at other times was of the viridans type. The other type of streptococcus did not occur in pairs and also hemolyzed blood. Neither of these types, and many strains were tested, altered inulin, nor were they dissolved by bile, nor could

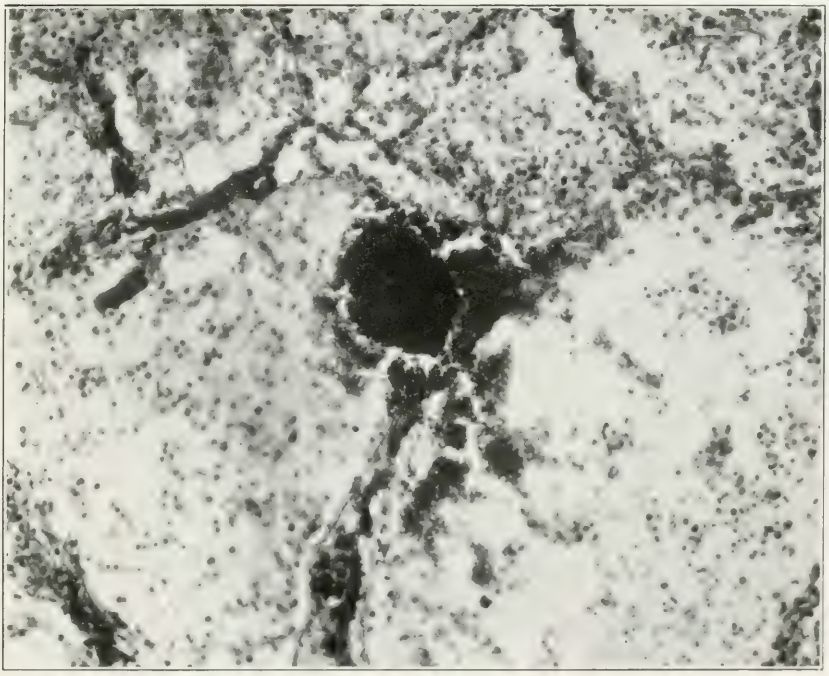

FIG. 1. Early changes in lung, showing intense congestion, character of exudate, and thrombus in blood vessel.

a capsule be demonstrated. Of twenty-two post-mortem cultures of the lung, twenty showed a diplostreptococcus of the hemolytic type, and two a pneumococcus, while a similar number of post-mortem blood cultures was sterile. In a series of forty-seven cases serum was obtained and its agglutinating or precipitin power in dilution of one in seventy was tested against a 300 million per c.c. suspension of (*a*) influenza bacilli, (*b*) both types of streptococcus, (*c*) the pneumococcus types, and (*d*) staphylococci. Agglutinins or precipitins could be demonstrated as follows: influenza 0; streptococcus 20. No action was observed in 27 instances.

The pathological changes in the tissues throw some light upon the clinical signs and show the violence of the infection. They are much better seen in the accompanying photomicrographs than they can be described, and rather than go into great detail an attempt will be made to construct the histology of the course of the disease in the various organs. In the initial stages in the lungs all that can be demonstrated is a most intense congestion proceeding to the point of rupture of many of the smaller blood vessels and the extravasation of blood into the alveoli. Within a

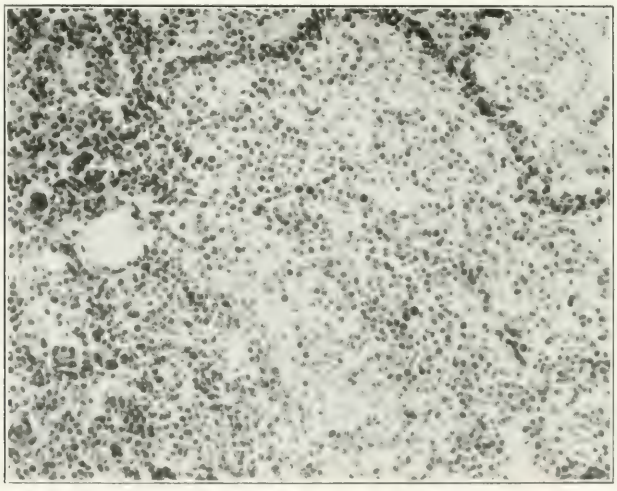

FIG. 2. Later changes with cellular exudate in the alveoli.

few hours there next appears in scattered areas a hyaline or homogeneous exudate in the alveoli, the congestion persisting, and not until the third or fourth day does a distinct cellular exudate appear, and with it, in many cases, the typical fibrin. The lung is not diffusely involved; the lesions are focal and of the bronchopneumonic type. Later thrombi form in the larger vessels and actual necrosis and abscess formation of the multiple type occur. Grossly the lesions correspond to the microscopical

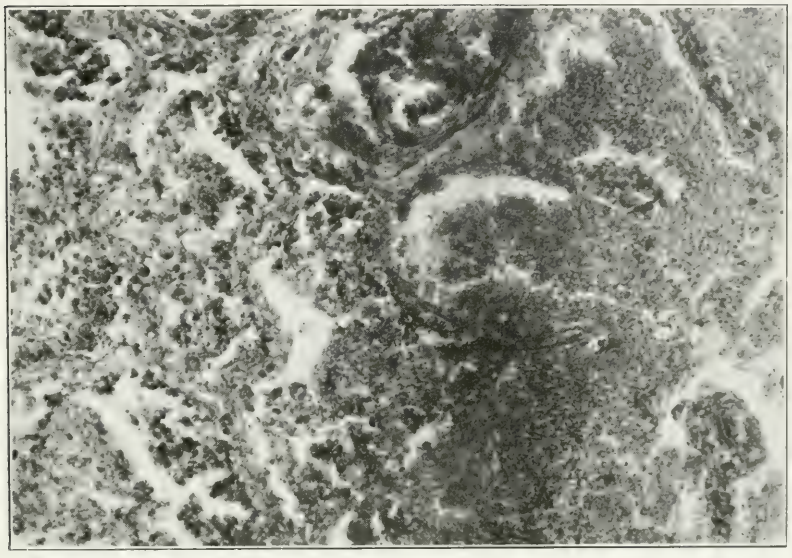

FIG. 3. Late stages, degeneration of lung tissue with abscess formation.

picture with coin-like or more diffuse areas of infiltration found most usually in the posterior and basal portions. One characteristic is the blue color of the infiltrated areas.

With these changes in the lung the effect of the toxin is also visible in other organs. Thus the cardiac muscle becomes pale and soft and microscopically emboli are demonstrable with alterations ranging from abscess formation to in one case coagulation necrosis of a rather extensive area of muscle. The liver shows at first a cloudy swelling with peculiar fatty areas of irregular

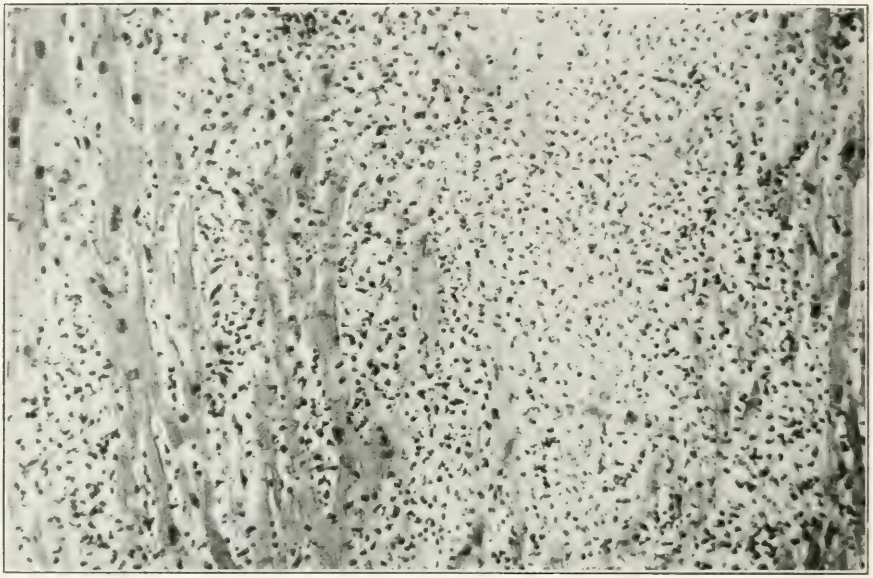

FIG. 4. Embolic abscess in the heart muscle.

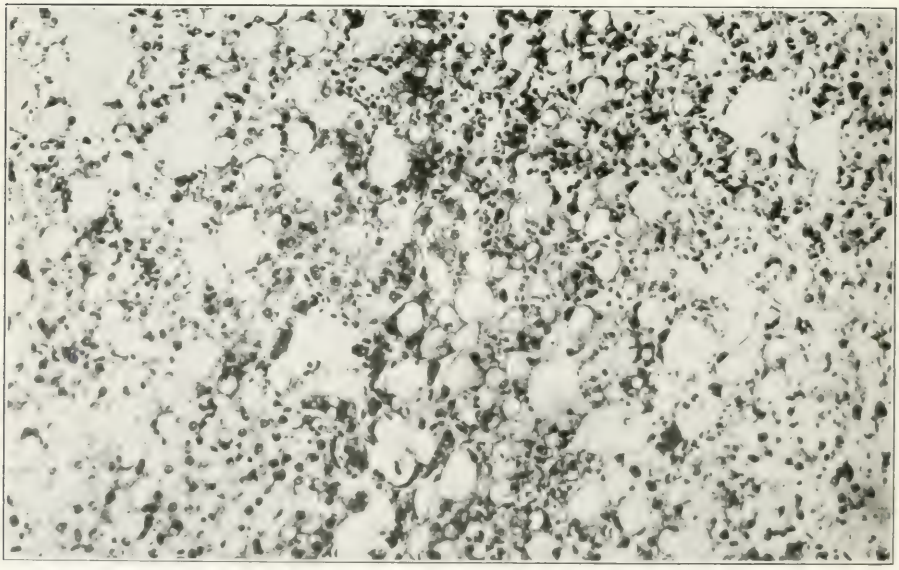

FIG. 5. Extensive destruction of liver.

shape situated beneath the capsule. This fatty degeneration may proceed to such extensive liver destruction as to suggest phosphorous poisoning. Even when these changes are not so marked the cells about the central portions of the lobule often show rather extensive bile pigment deposits and an intense congestion. In the gross the liver is usually large. The spleen is fairly uniformly small and intensely congested, in keeping with the leucopenia so generally present. The kidneys show their most marked changes in the cells of the tubules, which are degenerated, and the tubules are often filled with granular debris. In a few instances the adrenal cells of the cortex have also shown a cloudy swelling. The changes in the nervous system have not been observed, since no opportunity has been given to open the skull in our autopsies.

THE ETIOLOGY OF "INFLUENZA"

ANNA W. WILLIAMS, M.D.

Bearing in mind the characteristics of each of the groups of microörganisms to which the organism belonged that had been described as the possible exciting factor in a series of cases in different parts of the world, we, of the Bureau of Laboratories of the Health Department, chose our culture media and methods of obtaining material, as far as we could judge from the meager reports, so that we might be able to detect any or all of the microorganisms described or any others of significance with the exception of a filtrable virus.

We came to the conclusion that a study of the existence of a filtrable virus, to be worth anything, involved such controlled handling of human tests that it could be done best in camps with volunteers—therefore, we decided to leave it to the camp workers. So far the few experiments made in this country for detecting a filtrable virus have been negative.[1] In France, two insufficiently controlled experiments have been reported.

[1] J. J. Keegan: *Jour. Am. Med. Assn.*, 1918, lxi, 1051.

We began our study of this epidemic about the middle of September. The contagious disease hospitals of New York City received groups of sailors from the Navy Yard in this vicinity so that we had the opportunity of studying a number coming down with the disease. At about the same time we also obtained material directly from the receiving ship in order to get some cases in the earliest hours of infection. We made a study as well of some early and later cases from a camp in this vicinity.

At first we made a study of cultures from different parts of the naso-pharynx (nose, tonsils, entrance of posterior nares into pharynx) and from the sputum (secretion from the deeper air passages cannot be obtained early in the disease). We soon found that we obtained cultures with less contaminations by the ordinary mouth organisms when we took them from the pharynx at the entrance of the posterior nares by means of a turned swab protected by a West tube, in the same way that cultures are obtained for the determination of the presence of meningococcus. We therefore took all our later cultures in early cases only from this area, and called them naso-pharyngeal cultures. Our second best results were from the tonsils. Cultures from the sputum in these early cases were very unsatisfactory, since many mouth organisms were present and the influenza bacilli were apt to be missed. Of course sputum from the deeper air passages obtained fresh should contain many of the organisms, especially in those cases where the infection goes down or begins deeper than the naso-pharynx, unless the influenza bacilli cling to the mucous membrane.

We chose media for growing these cultures which we had found best for the development of influenza bacilli, of hemolytic streptococci, of pneumococci, of the streptococcus viridans group, and of meningococcus-like organisms. We arranged our record cards so that we could note the presence and number of these organisms and of any other microörganisms that might either be in great abundance or have peculiar characteristics. We also examined cultures under partial anaërobiosis in our hunt for unusual organisms, with negative results so far. Our general method of making this study may be described as follows:

At the bedside:

1. Cultures are taken from the nasopharynx with a swab protected by a West tube.
2. A small area is inoculated on one side of blood-pour[2] vitamin agar plate.
3. On a blood-drop agar plate (a drop of blood placed on one side of veal agar plate) a similar small area is inoculated with the same swab.
4. The swab is then rubbed around in a tube containing blood broth (4 per cent. whole blood) and afterwards removed.

The two plates and the blood broth tube are carried to the laboratory.

At the laboratory:

1. With a sterile platinum loop, using vitamin agar, another blood-drop plate is inoculated from the inoculated area of the first, streaking out over the entire plate many streaks from the inoculated area.
2. The other two plates are then streaked in the same manner.
3. Two blood-pour-agar plates are made, inoculating while the blood-agar is still melted (at 45° C.) with one loop and 0.2 c.c. respectively from the blood-broth tube inoculated from swab.

In twenty-four hours the influenza colonies appear (1) on blood-pour-agar plates to the naked eye as minute dew-drop colonies, and (2) on blood-drop-agar plates under the microscope, magnified 100 diameters, as small whitish areas embedded in the red blood cells. The pneumococcus and streptococcus show a greenish rim; the blood cells are not so used up. The influenza colonies are most typical on this plate. They often show a characteristic "heaping" in the center. When surrounded by blood cells the margins of the colonies often appear irregularly scalloped, but on the edges of the blood mass the free margins of the colonies are usually entire, with an occasional indentation. It is difficult, however, to focus on a definite marginal line, as the margin seems to vanish suddenly on focusing. The third dilution—the blood-drop-vitamin-agar plate—often shows very large colonies of influenza bacilli. Transplants are made on glycerin-veal-blood agar (chocolate medium). The formula of the latter is the following:

Chocolate medium:

Veal agar neutral to phenolphthalein.
Glycerin 5 per cent.
Whole blood (horse or other), 5 per cent.
Add blood to glycerin-veal-agar just after latter has been removed from the sterilizer (at about 90° C.). The formula for vitamin agar is as follows:
Beef heart, fresh, trimmed free from fat and tendon, and finely chopped.
Peptone, Difco 1.5 per cent.
Sodium chloride 0.5 per cent.
Eggs and tap water.

[2] We used citrated horse blood for all of this work.

PROCEDURE A.

Beef heart	500 Gm.
Tap water	500 c.c.
Egg well beaten	1
Peptone	15 Gm.
Sodium chloride	5 Gm.

Heat in water bath, double boiler, or over open fire with constant stirring until color changes to brown (at about 68° to 70° C.). *Do not use cheesecloth.*

PROCEDURE B.

Agar	15 Gm.
Tap water	500 c.c.

Heat to dissolve agar, either in autoclave or over open flame. If the latter, make up the water lost by evaporation. Do this by weight. When agar is completely dissolved, cool to 70° C. and add mixture. Set reaction 0.4 acid to phenolphthalein (room temperature titration) by adding normal sodium hydroxid, or make slightly alkaline to litmus paper. Place mixture in autoclave and heat for one half hour at fifteen pounds pressure. Remove kettle carefully without disturbing contents and set aside to solidify. The meat particles, etc., settle gradually to the bottom. After the agar hardens completely, turn it out in mold form and trim off the layer containing the sediment. The remainder of the agar is fairly clear. Melt and tube or bottle. Sterilize for one half hour at fifteen pounds pressure. In not all cases even with the best medium do the colonies of influenza bacilli appear in twenty-four hours. Negative plates should be returned to the incubator and examined again after forty-eight and seventy-two hours before being discarded as negative.

We examined about 200 cases diagnosed clinically as influenza in from the first to the sixth day of the disease. In all of these cases we found that the earlier the case was cultured the more constantly did we find large numbers of influenza bacilli in the naso-pharynx. No other picture was so constant, though we did find a high percentage of meningococcus-like organisms, and of streptococci belonging to the viridans group.

We have had two groups of cases which bore out this picture strikingly.

The first group was in a children's home where some nurses, who had been sent out to a blown-up munition plant to help the sufferers among whom many cases of influenza developed, came down with the disease just after they returned to the Home. An

epidemic swept over the institution in quite a virulent form. We were able to culture many of the cases in the first day of the disease. These all showed abundant, almost pure cultures of influenza bacilli, and 98 per cent. of the others (forty-eight cultures in all) showed the bacilli in large numbers. Among nine cultures taken in the Admitting Ward which was not kept strictly quarantined, four showed influenza bacilli, one from a case having a nasal discharge contained many bacilli, and the others very few. Any deduction drawn from the high incidence of the influenza bacilli in the typical cases of this group was vitiated by the fact that there had been a number of cases of pertussis in the institution during the summer in which disease the percentage of influenza bacilli is very high.

The other group consisted of eighty Marines who came from the south in a body, and who just as they arrived in New York all came down with the typical "influenza." They were sent to the hospital immediately. We cultured thirty of them and they all showed large numbers of influenza bacilli, in almost pure culture in many of the cases.

In this group there had been no pertussis and no measles, in both of which diseases many influenza bacilli are usually found.

Our striking control so far is from a Girl's Home containing 500 inmates where no "influenza" cases had occurred. Thirty-four cultures were taken from different groups of girls, and in only two, one of whom had a "slight cold," and had been kept isolated, were influenza bacilli found.

Since this examination was made we have examined a preventorium where the inmates had not been kept strictly isolated from the village in which there had been a number of cases of influenza, with one typical case and the usual number of short fevers. Here we found that 33 per cent. of the cultures contained influenza bacilli.

We were able to make a bacteriologic examination of thirty autopsies, the results of which are given briefly below.

In twenty-four cases we found influenza bacilli in the lungs, in five of which they were in pure culture. In twenty-six out of

twenty-seven tracheas in these cases we found influenza bacilli in large numbers. We found pneumococci (mostly type IV) in fifteen lungs, streptococcus hemolyticus in nine, Gram-negative in one, Staphylococcus in two and a small Gram-negative bacillus in one. We thus see that the influenza bacillus was found more frequently than any other one organism. But whether or not it was the initiating cause or only a more frequent secondary invader than the other bacteria we cannot say.

All of our results in regard to the incidence of the influenza bacillus may be tabulated as follows:

B. Influenzæ

Group.	Present.	Absent.	Per Cent.
Earlier hospital cases	160	40	80
Marines	30	0	100
Home for children	47	1	98

Controls

	Present.	Absent.	Per Cent.
Nurses—contacts	4	6	40
Nurses—non-contacts	1	7	9
Measles	4	2	67
Admission ward	4	5	41
Home for girls	2	32	6
Preventorium	14	25	33

Autopsies

	Present.	Absent.	Per Cent.
Lungs	24	6	80
Pure cultures	5	25	17
Tracheas	26	1	96

Just as soon as we began to find the influenza bacilli in such large numbers in the early cases and in pure cultures in the lungs, and in very large numbers in and between the cells in direct smears from most of the tracheas examined at autopsy, we came to the conclusion that whether or not this bacillus is the initiating cause of the present epidemic it is evident that it has a marked pathogenicity and this seemed to be sufficient reason for us to

make as thorough a trial of the effect of an influenza bacillus vaccine on the prevention, if not of the pandemic, at least of a number of the cases of infection, with this bacillus.

The problem of producing immense quantities of vaccine, of determining the best dosage, the time for immunity, if any, to be established, of getting the proper controls in communities not yet infected, and above all of keeping a good record and gathering and analyzing the data was one of the most important and staggering of problems.

We began the use of our influenza vaccine after the pandemic had already started and some groups were not vaccinated until after the peak of the pandemic had been passed, so unless we could get sufficient control cases, that is, presumably susceptible unvaccinated cases, we could arrive at no conclusion with regard to the efficiency of the vaccine. It is strange how difficult it has been for us to get the right kind of contacts. The reports from the use of our vaccine are being analyzed now and it is already apparent that the majority of them are lacking in many essentials.

The intelligently controlled use of the vaccine might have given us strong evidence in regard to the etiologic relationship of the influenza bacillus to the present pandemic; as it is, unless the finished reports prove to be better than they now promise, we must wait for such evidence as may be gathered from a serologic study of the various strains of the influenza bacillus isolated by us from the typical cases and from control cases in this study. We are carrying on a serologic study of influenza bacilli isolated for the purpose of gaining evidence as to the presence of a pandemic strain.

CONCLUSIONS

The evidence in favor of the influenza bacillus being the initiating cause of the present pandemic of "influenza" is not strong enough to constitute proof.

No evidence as strong has been brought forward in favor of any other cause.

Whether or not the *B. influenzæ* is the initiating cause, it has

given evidence that it has a marked pathogenic action and the study of a vaccine to prevent this action should be continued until we are at least sure that it can give us no positive results.

EXPERIENCES WITH VACCINE IN AN INFLUENZA EPIDEMIC

EDWARD G. CARY, M.D.

There were 500 corps men stationed at General Hospital No. 1 when the influenza epidemic started, and of these fifty-one (10.2 per cent.) were admitted to the hospital during the period from September 26 to October 2.

On October 2, 171 men of this detachment were inoculated with a commercial vaccine made up of a mixture of influenza bacilli, pneumococci, streptococci, staphylococci, micrococcus catarrhalis, and Friedländer bacilli. Of this group thirteen (7.6 per cent.) were admitted to the hospital during the period October 2 to November 1. Of these thirteen cases, however, nine were admitted within forty-eight hours after vaccination, and in all probability were in the incubation stage of the disease at the time of inoculation. The remaining four cases really represent the true incidence of the disease, 2.3 per cent., for the period from October 5 to November 1.

Of the 283 men remaining in the detachment who were not vaccinated, thirty-seven were admitted to the hospital with influenza during the period from October 2 to November 1, an incidence of 13 per cent. If we compute the incidence in this group for the period from October 5 to November 1, we find an incidence of 6.5 per cent. (17 cases). The unvaccinated showed a somewhat higher percentage of incidence than the vaccinated during these periods and at first glance it appears that there was a fairly definite benefit from the vaccination. A further examination of the admissions shows that forty-eight hours after one series had been inoculated there had been a very sharp de-

crease in the rate of incidence, and that four days after that time marked practically the end of the epidemic among the corps men. In view of these facts it seems very doubtful whether there was any real benefit derived from the use of the vaccine as far as this group is concerned.

In another series comprised of 136 nurses there developed during the period from September 26 to October 2 fourteen cases, an incidence of 10.3 per cent. On October 2 forty-seven of this group were inoculated with the same vaccine used for the corps men. Of these forty-seven vaccinated nurses five developed influenza by November 1 (10.6 per cent.). Of the seventy-five who were not vaccinated twenty developed influenza (26.6 per cent.) and of these twenty, four died. In this series there was not so sharp a peak to the incidence curve as with the corps men, but the cases were well distributed over a period of ten days following the administration of the vaccine to the one group. As far as can be judged by the groups of vaccinated and unvaccinated nurses there was considerable benefit derived from the use of the vaccine—this benefit is represented by an incidence of 10.6 per cent. in the vaccinated, compared to an incidence of 26.6 per cent. in the unvaccinated.

In none of the vaccinated series did a death occur.

Treatment of actual pneumonia cases with intravenous administration of vaccine was tried. The vaccine used was made up of *B. influenzæ*, 100 million; pneumococcus I, II, and III, 33 million each; *Staphylococcus aureus*, 100 million; *Streptococcus hemolyticus* 100 million. The influenza bacillus, streptococcus, and staphylococcus were from actual cases of pneumonia that developed during the epidemic; the pneumococci came from stock cultures.

In treatment the procedure was to give 0.5 c.c. of the above mixture intravenously the first day and to follow it twenty-four hours later with a 1 c.c. dose of the same mixture. Depending upon the severity of the reaction the injections were given at twenty-four hour intervals until in some cases four or more injections were given. As much as 5 c.c. was given at a single in-

jection without a reaction in some cases, while others showed a very sharp reaction to the second dose of 1 c.c.

Of 86 cases of pneumonia that were not treated with vaccine 32.5 per cent. died. In this series all who died were admitted before October 3.

In a second series of 216 cases of pneumonia treated with intravenous vaccine, nineteen died (8.8 per cent.), and of these nineteen the admissions dated from September 28 to October 21, with the greater number of admissions before October 4.

In this second series it is possible that we were dealing with a less virulent infection or a more resistant type of patient, but in spite of this it is hard to believe that the vaccine was without benefit in many cases in which there was a sharp reaction following the second dose, with a subsequent drop of temperature within twelve to twenty-four hours to practically normal, and a prompt recovery of the patient. A few responded to the first inoculation with reaction and benefit. In many cases neither reaction nor benefit seemed to result.

Discussion:

Dr. Moschcowitz: I should like to ask Dr. Cary what kind of vaccine he used.

Dr. Cary: We used a mixed vaccine. However, I do not know that it makes a great deal of difference what sort of vaccine you use. Ours was a combination of influenza bacilli, staphylococcus, hemolytic streptococcus, and three groups of pneumococcus. As I said, the influenza bacillus, hemolytic streptococcus, and the staphylococcus were isolated from autopsied cases. One reason that we used these is that we are dealing with pneumonias due to infective organisms that are not usually met. In these cases we seem to have a lot of infection with streptococcus, some with a staphylococcus, and sometimes with Group I, II, and III pneumococcus. By combining these, if you can get at one time immunity to pneumococcus I, II, and III, I do not think it a bad idea. The quantities are 100 million of each, with the exception of the pneumococci, of which there are 33⅓ million of each group, giving a total dosage of 400 million per c.c.

Dr. Ewing: I should like to ask Dr. Williams if she has made any study of smears from different portions of the lung at autopsy, that is, from the bronchi, trachea, and the diffuse pneumonias, and also what organisms she gets in the diffuse pneumonias. She mentioned observations on that point, but as I recall, she did not state just what organisms were recovered in these diffuse pneumonias.

Dr. Williams: I gave that in my first report. In the diffuse pneumonias the majority of cases showed pneumococcus with the influenza bacillus. We examined a number of cases and two thirds of them showed pneumococcus and one third streptococcus hemolyticus. We did not get any green streptococci at all in these lungs. We had two lungs showing pure streptococcus hemolyticus, and we found staphylococcus with the pneumococcus in two cases. In one case a meningitis-like organism was associated with the pneumococcus. In one case we got an interesting organism which looks very much like the influenza bacillus, but it grows on plain agar, that is, it is not a hemoglobinophile. It produces colonies similar to the influenza colonies, and as it grows more rapidly than the influenza bacillus, we only found it in the first fishings from the lung cultures. At first I thought that this was the influenza bacillus, and I went back to the lung which had been in the ice-box about a week, and made more dilution plates on which developed many influenza colonies among the others. From the trachea and bronchi of all but one of our cases examined—twenty-six out of twenty-seven—we obtained many influenza bacilli.

Dr. Ewing: I have been very much interested in the pathologic anatomy of the influenza pneumonias. At the Army Medical Museum in Washington there have been a considerable number of lungs sent from camps in different parts of the country. These lungs, together with the patients' histories, were sent for examination, so that we have seen the lesion as it developed in Washington, at Camp Meade, at Devens, at Mills, at Wheeler,—in fact, from the fairly well distributed camps in the eastern part of the country. They look pretty much the same. In the early part of the epidemic these lungs were mostly the blood-dripping organs which are so characteristic of the more severe type of cases. When one examines sections of organs in the condition in which these were received it is very unsatisfactory, because the whole lung is practically a mass of coagulated blood. Then there is another group of cases which I think came a little later in the course of the disease, in which there is the same diffusion of blood, only there are throughout the lung minute pin-point lesions, which prove to be a catarrhal suppurative and usually necrotic bronchiolitis. Microscopically there was a very general catarrhal suppurative bronchiolitis, also seen in the earliest cases. In a good many of the lungs one would find that this lesion was wiped out, as it were, by a diffuse pneumonia. The pneumonia had various characteristics. There was seldom a straightforward lobar process, although in a certain number of cases there would be an exudate which wiped out the bronchiolitis and gave the picture of ordinary lobar pneumonia. But in the majority of cases the lung was not increased in size; there were areas of necrosis, and often an interstitial purulent lymphangitis. The lesions were similar to those which we have seen many times before, especially last winter. There were about four different types of lungs: one presented the bloody exudate; others showed irregular necrotic pneumonia, and others the lobar areas. I have tried to find a uniform relation between the bacteriological reports in these cases and the pathological condition, but I have not succeeded. I suppose in the rush of the work at the different camps it was impossible to make satisfactory

bacterial examinations. I have also been very much impressed by the specific character of the changes in the other organs. From the first I noticed that there were these ordinary secondary pneumonias which I had seen before, and as the material kept coming in from different localities, my impression grew that the pathological anatomy approached more and more the lesions seen in civil life, and especially those which we saw last year in the camps. About two weeks ago I undertook to compare a series of lungs received last spring and winter from all over the country with the lungs which we have been getting in the last six weeks, and found that there were a great many alike. I found it possible to duplicate practically every one of the recent types with those received last year. There is a little more hemorrhage in the early cases, and more rapid death in the earliest of the epidemic cases of this year than in any I have been able to trace among the pneumonias of last year. From this comparison I have been led to conclude that the disease which we have been dealing with this fall is only a more virulent form of the disease which swept over the camps last year. Another significant matter is the change in the character of the pneumonias which has already occurred in the present epidemic. On September 12 before there was any definite word about influenza (I think it had possibly just reached the newspapers) I saw in Washington what I now believe to be a typical case of influenza pneumonia, and while I was not particularly thinking about influenza, I said at the time that this might be such a case. From that time on the influenza increased in that hospital and they had a very severe epidemic. First the severe cases came, but as the epidemic went on, it is very striking to see how the so-called peculiar new features which many said indicated a new disease were replaced by less severe and more ordinary symptoms of severe pneumonia, and it is especially striking how they approximate the lesions which we saw last year.

Dr. Park: One point I should like to speak of is one that is familiar to all who have read the abstracts of the mass of literature in the Journal of the American Medical Association, and that is that we are simply bewildered by the absolutely contradictory reports on the bacteriology. I think I am stating Dr. Williams's opinion (as she has been doing the work) when I say that we cannot help but feel that, whether the influenza bacillus is the etiological factor in this epidemic, it must be present in the severe cases, and that when it is not found it is because the best way of finding the bacilli has not been used. We have had the experience a number of times of going to a camp or hospital where the organisms were not being recovered in a large percentage of cases, and in the swabs or the autopsy material obtained from those same cases, Dr. Williams would get the bacilli. Also in our very earliest severe cases Dr. Williams did not recover influenza bacilli in every instance, while she never misses them now. This change she thinks is because of her increased familiarity with small but essential points. We have had material from Boston, from Upton, from New York, and from ships, and in all of this we found the same predominance of influenza bacilli. Workers from other places who have come to our laboratory have usually felt that they have missed them in their examinations from neglect of some of the little but very essential points. Therefore it seems to me that those who are

going on to make cultures in the remaining cases ought to use the best possible technique, and if any do not get positive results, it would be very desirable to send the material to us and see if we can find the organism where they failed. I know of one hospital that reported an outbreak of influenza in which the acting bacteriologist was unable to find the influenza bacillus, and Dr. Hatfield went there and found an abundance of bacilli in all the cases. Dr. Ewing's conclusion that because these cases present similar pathological findings to those in the camps last year, that therefore the infecting agent in this epidemic is probably the same as spread disease in the camps last year, does not seem to me to be correct. I think this epidemic is an absolutely different thing,—so different that I assume we have to deal with an organism with which we are not familiar, or with some new strain of a familiar organism. A real epidemic must be due to one type of organism. There may, of course, be two epidemics at the same time, as for instance, an epidemic of typhoid fever and one of dysentery. Certainly last year healthy people did not frequently contract the disease from simple contact. It usually followed measles or some other infection. The almost constant presence of influenza bacilli in cases of whooping cough and measles makes it difficult to decide whether the presence of influenza bacilli in the epidemic cases really has the importance that it seems to have. That is the reason why we and the workers at the Rockefeller Institute and elsewhere are trying to see whether the bacilli from the cases belong to a single strain. I think the work is tending to show that we have isolated in at least a few cases different strains. I am sure that the cases which we have gone over show the influenza bacillus to be present regularly and to be the dominant cause of the lesions in many cases. The prevalence of influenza bacilli in healthy persons makes it extremely difficult to decide whether the bacillus is the cause of the epidemic or only a secondary invader which is more important than the primary one. As more than half the cases do not show pneumococci, and more than half do not show streptococci, these cannot be the cause of the epidemic, but must be regarded as secondary infections.

Dr. Moschcowitz: It strikes me as rather curious that if, as Dr. Ewing believes, the pneumonias in this epidemic are identical with those seen in the camps last winter, there should be the peculiar absence of pleural infections in this epidemic as contrasted with the amazing incidence of pleural infections at that time. This would tend to disprove what Dr. Ewing has said. I wonder if there is anyone here who remembers the epidemic of 1899, and if they recall whether the pneumonia was similar to that found now.

Dr. Libman: In 1901 I saw lungs which had the characteristics of the pneumonia seen last year: the peculiar greyish lesions which so many people took for tuberculosis. I remember one of those lungs which I worked over for a long time trying to prove whether it was tuberculous or not. That type of pneumonia is a very well known one and has been apparently entirely overlooked despite the fact that it has been very well described in the text books. As Dr. Ewing has said, the cases this year seem practically to correspond to those seen in previous years. The pneumonias seen now (at the

end of the epidemic) are mostly basal; in the early part they were found very frequently beginning in the upper part of the lower lobe. The cases we are seeing now are apt to be accompanied by pleural infections, in contradistinction to the early cases of the epidemic. One interesting point about the relationship of the influenza bacillus to the disease is this, a point that Wassermann brought out in 1901 or 1902; He states that following one of the large epidemics in which the influenza bacillus was found in most of the cases, in the next two years there were smaller epidemics, but that the influenza bacillus could not be found in the latter cases. He states that possibly the people in the earlier epidemic had gotten up an immunity and that these organisms were present early in the disease and were killed off rapidly, and then that these secondary organisms got the upper hand. That view may be right and it may be wrong.

Dr. Ewing: I do not pretend that pathological anatomical studies alone are going to decide this question. I only say that the material I have seen indicates to me pretty clearly that the anatomical lesions of these pneumonias which we are getting now are indistinguishable from those we got last year, but I recognize that all of this must be controlled by bacteriological studies. Up to to-day it seems to me that the bacteriologists have not reached a decision. Until they do, I do not think it wise to ignore the pathological anatomy.

Dr. Williams: Were bacteriological studies made of those lungs in the hospitals in Washington, and if so, what did they show?

Dr. Ewing: The Walter Reed Hospital made very careful studies, and they found a high proportion of influenza in the lung. At Meade it was mostly the streptococcus. There were a good many types of secondary infections with diffuse pneumonias which followed the influenza.

Dr. Williams: I understood that everybody agreed that the majority of the deaths were due to secondary infections.

Dr. Cary: In regard to this question of pleural infection: out of twenty-four autopsies I found that nearly all had a pleural infection; there were two or three that had fibrous pleurisy and the matter of acute infection was not definitely made out. Nineteen or twenty showed some pleural involvement, ranging from an inflamed pleura to an empyema. There were five empyemas. But there was some pleural involvement in practically every case, and the infective organisms found in the majority of cases were various types of streptococcus, either hemolytic or viridans. There was one pneumococcus. In regard to the infective organisms in the lung, I found a preponderance of streptococcus. As I said, some of these were hemolytic, two of them were of the viridans type, but the larger number were an indefinite type of streptococcus. This was the predominating organism. In culturing the lungs I have not cultured the various parts, but I think if this were done, you might get various organisms. Such a condition has been reported to me as having existed in the experience of the men in France. Cultures from different parts of the same lung gave different types of pneumococci, and also streptococci. In other words, it is a mixed infection.

Dr. Libman: I just want to say again that clinically the cases early in the epidemic did not present evidence of empyema. Lately we have been getting it. I am sorry that I did not bring along a letter from Major Baehr. He had seven to ten autopsies a day, and he comments on the practical absence of empyema. I think Dr. Cary must have encountered an unusual incidence of this complication for the early part of the epidemic.

Dr. Cary: The autopsies cover practically the whole of the epidemic,—from October 1 to November 1.

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